GRASSLAND INVASION BY NON-NATIVE GRASS SPECIES: ECOLOGICAL ISSUES OF MULTIPLE SPECIES AT MULTIPLE TROPHIC LEVELS

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Abstract: Invasion of native grasslands by non-native grasses is of great economic and ecologic concern. Non-native grasses have potential to alter ecosystem functions, habitat quality, disturbance regimes, and feedback loops. These alterations can have bottom-up or top-down effects that may negatively influence grasslands at multiple trophic levels. I conducted three integrated studies to assess non-native grass invasion: 1) on the competitive interactions between native vs. non-native grass species with community-level implications; 2) possible utilization of allelopathic biochemicals as an invasion mechanism; and 3) possible alterations in the small mammal communities with implications for ecosystem-level function. My first study indicated that both invasive and native species varied in mycorrhizal dependency along a continuum from obligately to facultatively dependent. Native species biomass production was consistently reduced when planted into 'away' soil, as compared to 'home' soil. Increased biomass production of native grasses was consistently observed following additions of native prairie soil to steam-pasteurized soil from the invaded sites, indicating invasive feedbacks may occur through alterations in biotic communities. My second study indicated that *Bothriochloa spp.* may gain a competitive advantage through the use of allelopathic biochemicals. However, it is unclear if these allelopathic effects directly hinder competitors, or indirectly hinder competitors through alterations of soil microbial communities. Determination of allelopathic biochemicals was not definitive. Data from my third study indicate that invasion of *B. ischaemum* into the native grasslands lowered all abundance metrics for deer mice (*Peromyscus maniculatus*), while increasing all abundance metrics for hispid cotton rats (*Sigmodon hispidus*), as compared to native grassland controls. Species-specific models show cotton rats select vegetation that supplies aerial predator avoidance and deer mice select habitat that increases foraging efficency. Alterations in these small mammal communities may have profound effects on ecosystem functioning. Our research indicates non-native grass invasion alters native communities on multiple trophic levels. I propose management practices for restoration may be most successful if determined on a species-specific and site-specific basis, as different species appear to use different mechanisms for successful invasion into native prairies.

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CHAPTER I

ENCROACHING AND INVASIVE WARM-SEASON GRASSES REDUCE NATIVE GRASS PRODUCTION: CONSEQUENCES FOR RESTORATION

ABSTRACT

Many invasive species of the southern and central Great Plains native grasslands were introduced to reduce soil erosion or increase forage production on marginal grasslands. Recent studies have indicated arbuscular mycorrhizal (AM) fungi play an important role in restoration success following invasive plant species eradication. We conducted two greenhouse studies: the first assessed mycorrhizal dependence of three warm-season invasive grasses, the second examined 1) mycorrhizal dependence of four native grasses; 2) native seedlings growth in soil collected from areas dominated by native grasses ('home') compared to soil collected from areas dominated by invasive grasses ('away'); 3) the influence of native microbial communities added to 'away' soil; and 4) native seedling growth in steam-pasteurized "home' and 'away' soils with native soil amendments. Our results indicate that both invasive and native species varied in mycorrhizal dependency along a continuum from obligately to facultatively dependent. Native species biomass production was consistently reduced when planted into 'away' soil, as compared to 'home' soil. Increased biomass production of native grasses was consistently observed following additions of native prairie soil to steamed soil from the invaded sites, indicating invasive feedbacks occur through alterations in biotic communities. However, our data also indicate

suppression of native plant growth appears to include other mechanisms, such as production of allelopathic chemicals. We propose management practices for restoration may be most successful if determined on a species-specific and site-specific basis.

INTRODUCTION

Biological invasion is an ecological term that refers to the process by which a new species enters a native biological community, reproduces, and displaces native species, resulting in economic or environmental impairment. While this process generally refers to non-native or introduced species, native species can also spread beyond their native range, resulting in adverse effects on native ecosystems. Typically, expansion of non-native species is referred to as invasion, while expansion of native species is referred to as encroachment (Davis et al. 2001). Expansions of native or non-native species have been reported to alter native species biodiversity (Heywood 1989), disturbance regimes (D'Antonio and Vitousek 1992), and ecological structure and functioning (Chapin et al. 2000). Biological invasion by non-native plant species has been cited as a major aspect of global change (Vitousek et al. 1997) and one of the major causes of native grassland loss (Watkinson and Ormerod 2001).

Lockwood et al. (2007) defines biological invasion as a three step process: arrival, establishment, and spread. Many invasive species of the southern and central Great Plains are non-native grasses that are or have been seeded to reduce soil erosion and increase hay and forage production on marginal or deteriorated rangelands (Harmoney et al. 2007). However, the rapid spread of these grass species into native grasslands is causing ecological and economic concerns (Harmoney and Hickman 2004; Hickman et al. 2006; Fulbright et al. 2013). Previous research assessing competitive abilities of invasive or encroaching grasses (hereafter referred to as invasive grass species) has focused on propagule availability or aboveground plant traits of the invading species (e.g. Tognetti and Chaneton 2012). As a result, we know considerably less about

invasibility as an emergent property of the comprehensive plant-soil interactions or the factors influencing these interactions. However, above- and belowground communities are inextricably linked, and it is well documented that soil organisms play important roles in regulating ecosystem-level processes in native systems. Soil microbial communities can be beneficial by increasing mineral solubilization (Derylo and Shorupska 1992), nitrogen fixation (Albrecht et al. 1981; Reynolds et al. 2003), and increasing access to soil resources (Bever 2002). However, soil communities also harbor parasitic or pathogenic bacteria (Westover and Bever 2001), nematodes (Augspurger 1990), or fungi (Johnson et al. 1997; Denison 2000; Bever 2002). Additionally, plants can alter soil characteristics in ways that feedback to affect the performance of that species or other plant species (Bever et al. 1997). These soil feedbacks can play a major role in the success of invasive species, with individual studies showing both positive and negative feedbacks during invasion (Reinhart et al. 2003; Bever et al. 2010).

Recent studies have indicated arbuscular mycorrhizal (AM) fungi play an important role in plant invasions (Pringle et al. 2009; Jordan et al. 2012). AM fungi can contribute to plant soil feedbacks, as AM fungal taxa can exhibit host-specific growth responses (Bever 2002), and the benefits a given plant receives can depend on the identity of its AM fungal associates (e.g. Johnson et al. 2010; Hoeksema et al. 2010). Non-native invasive plant species, in particular, have been shown to alter the density and/or composition of the AM fungal communities, which may feedback and increase the subsequent spread of the introduced plant species (Bever 2002, 2003; Reinhart and Calloway 2006). However, the majority of invasions by non-native plants involve species that are functionally distinct from the dominant native species (e.g. Vitousek and Walker 1989; Orr et al. 2005; Batten et al. 2006) and this can include their response to AM fungal associations (Pringle et al. 2009; Seifert et al. 2009; Owen et al. 2013). Plants vary widely in their association with AM fungi, and a review by van der Putten et al. (2007) indicates that the majority of non-native species are non-mycorrhizal or less dependent on AM fungi than native

species. This lack of dependency may be a successful strategy of some invasive plant species. In contrast, the growth and fitness of other invasive plant species are highly dependent on AM associations and studies indicate there can be a promotion in plant invasibility through their association with native AM fungi (Shah et al. 2008; Shah et al. 2009; Wilson et al. 2012). Grasslands dominated by warm-season grasses, such as those in the central and southern Great Plains of the USA, may be particularly vulnerable to invasion by warm-season non-native grasses, as these species share the same ecosystem-level functional group as the dominant grass species. For example, old world bluestems (*Bothriochloa spp.*) have invaded the central and southern Great Plains, with native and non-native warm-season grasses being highly dependent on AM fungal associations for growth in grassland soils (Wilson and Hartnett 1998; Wilson et al. 2012). Wilson et al. (2012) reported an important mechanism for these species' successful invasibility may be self-facilitation through modification of the mycorrhizal associations, leading to positive plant-soil feedbacks.

Previous research has shown that species dependence on AM symbiosis plays an important role in warm-season grass invasibility (Wilson et al. 2012). Therefore, we conducted a greenhouse study to assess mycorrhizal dependence of three warm-season invasive grasses (two non-native and one encroaching native species) expanding into grasslands throughout the south-central and southwestern U.S. (Table 1). Based on previous research (e.g. Wilson and Hartnett 1998; Wilson et al. 2012), we hypothesized that these invasive warm-season grasses would be highly dependent on mycorrhizal associations to complete their life cycles in low nutrient grassland soil.

In a second experiment, we examined plant-soil feedbacks of the invasive grass species indirectly by assessing biomass production and AM colonization of native warm-season grasses planted into soil collected beneath invasive grass species (Table 1). We examined 1) the mycorrhizal dependency of four native grass species to AM fungi; 2) the ability of native

seedlings to grow in soil collected from areas dominated by native grasses ('home') compared to that of growth in soil collected from areas dominated by each of three invasive grass species ('away'); 3) the influence of native microbial communities added to each 'away' soil; and 4) the ability of native seedlings to grow in 'away' soils from which soil communities associated with the invasive were eliminated and native soil microbial communities were returned.

We hypothesized the mycorrhizal dependency of all four warm-season native grasses to be highly mycotrophic, based on previous research by Wilson and Hartnett (1998). We hypothesized seedlings planted into 'home' (native field soil) would produce greater biomass, compared to seedlings planted into 'away' soil (field soil collected from areas dominated by invasive grasses), as alterations in the soil microbial communities have been shown to hinder the growth of native grasses, due to negative plant-soil feedbacks established by the non-native grasses (Bever et al. 2002; Bever 2003; Wilson et al. 2012). Furthermore, we hypothesized that amending 'away' soils with native field soil inoculum would result in greater biomass of the native grasses, compared to growth in non-inoculated 'away' soils, and serve as a potential restoration tool. Finally, we hypothesized that if the feedback functions as an alteration in biotic communities, plant growth suppression would be alleviated through steam-pasteurization (eliminating altered soil microbial communities) and re-inoculation with freshly collected soil from non-invaded sites (re-establishing native soil microbial communities). Understanding how soil microbial communities, such as AM associations, influence plant invasions may be a critical aspect of the ecology and management of invasive plant species and the conservation and restoration of native grassland ecosystems.

MATERIALS AND METHODS

Soil collection sites

Soil was collected from Temple Ranch (Mosely, TX, Duval County, Texas 27˚ 58' 44.6" N, 98˚ 58' 13.6" W) and Hixon Ranch (Kingsville, TX, La Salle County, Texas 28˚ 23'01.45" N, 99˚ 12' 22.33" W), both private ranches located in south Texas. Both ranch sites are characterized by the invasive non-native warm-season grasses *Dichanthium annulatum* [(Forssk.) Stapf] (Table 1) and *Pennisetum ciliare* [(L.) Link] (Table 1). Temple Ranch also has problematic expansion from the native warm-season grass *Heteropogon contortus* [(L.) P. Beauv. Ex Roem. & Schult.] (Table 1). *Dichanthium annulatum* was introduced into the US in 1917 from areas of Asia and Africa for forage production and erosion control on marginal lands (Gonzalez and DallaRosa 2006) and is documented to occur in TX, LA, and HI (Gonzalez and DallaRosa 2006). *Pennisetum ciliare* was introduced into the US from Europe, Asia, and Africa in the 1930's for livestock forage (Griffin 1993), and is expanding its range in TX, CA, AZ, FL, HI, LA, MO, NM, NY, and OK (USDA 2012). *Heteropogon contortus*, native to southern and central U.S. and distributed worldwide, has been documented to be expanding its range in TX, AZ, FL, HI, NM, and is listed as a noxious weed in CA (USDA 2012).

Temple Ranch is primarily a wildlife operation that implements wildlife habitat management practices such as prescribed fire and mechanical brush control. The study site lies within the western Rio Grande Plains region on the Bordas Escarpment (Gould 1975). Mean annual rainfall is 76 cm per year (rainfall averaged from 1966–2000), with precipitation peaks in May and September (Norwine et al. 2007). Temperature fluctuates from an average high of 27.2° C in summer to an average low of 16.7° C in winter (temperature averaged from 1966–2000). Soils on the study area are classified as Czar Clareville soils, with a sandy loam surface texture (USDA 2012). This ranch primarily consists of mixed brush, native short and mid-grasses, and

forbs. *Dichanthium annulatum*, *P. ciliare*, and *H. contortus* are invading throughout the ranch in relatively small isolated monocultures (<0.25 ha).

Hixon Ranch lies within the western Rio Grande Plains region (Gould 1975). Soils on the ranch are part of the Bookout clay loam and Dilley fine sandy loam associations (USDA 2012). Annual mean precipitation is 62 cm per year (averaged from 1966–2000), with peaks in May and September (Norwine et al. 2007). Temperature fluctuates from an average high of 27.9° C in summer to an average low of 14.3° C in winter (temperature averaged from 1966–2000). Since the 1970s, the ranch has implemented extensive fire and mechanical brush control to manage for wildlife and cattle. *Dichanthium annulatum* and *P. ciliare* exist in monocultures across the area, nested within native grasslands.

Soil and seed collection and processing

Three replicates, of two 1 m^2 plots were established in areas dominated by *D. annulatum*, *H. contortus*, and *P. ciliare* at the Temple Ranch site. As a control, we established three replicates of two 1 m^2 plots in adjacent non-invaded grassland dominated by native warm-season grasses including *Chloris cucullata* [Bisch.], *Seteria vulpiseta* [(Lam.) Roem. & Schult.], *Digitaria californica* [(Benth.) Henr.], and *Pappophorum bicolor* [Fourn.]. Similar 1 m^2 plots were established at the Hixon Ranch site, with the exception of *H. contortus*, as this invasive species was not present at this site. Within each plot we collected soil (0 - 10 cm) from directly beneath the dominant grass species. Soil was then transported to Oklahoma State University, sieved to remove large debris and rocks, and homogenized. Soil collected from the same species at each site was also homogenized, as determination of general (not site-specific) trends in plant-soil feedbacks were the focus of this study. Half of the soil collected from beneath each grass community (three invasive species and native prairie control) was steam-pasteurized for 2 h at 80^{o}C and allowed to cool and equilibrate for 14 days. Before and after steam-pasteurization, but

prior to seedling transplantation, soil pH, plant-available P (Mehlich test 3), NH_4, and NO_3 (Table 2) were analyzed by the Oklahoma State University Soil, Water and Forage Analytical Laboratory, Oklahoma State University, Stillwater, OK. No significant differences were found between non-steamed (nonsterile) and steamed soil that had been allowed 14 days to equilibrate.

The invasive grass species were grown in soil collected from prairie dominated by the native grass species. The native species (*P. bicolor*, *S. vulpiseta*, *C. cucullata*, and *D. californica*) were grown in four soil sources ('home' soil collected from native prairie dominated by the specific study species and 'away' soil collected from prairie dominated by each of the three invasive grass species). The inclusion of the four selected native species planted into soil associated with multiple invasive grass species allowed for species-specific assessments.

Seeds of all native species were obtained from the South Texas Natives Ranch in Kleberg County Texas (Texas A&M University Kingsville, Caeser Kleberg Wildlife Research Institute, South Texas Natives, 700 University Blvd, Kingsville, TX USA 78363). These native seeds originated from cultivated stands of locally adapted ecotype seed releases. The seed of invasive grass species was hand-collected from patches adjacent to the soil collection plots at each study site. Once collected, seeds of all species were germinated in vermiculite and transplanted fourteen days (second-leaf stage) after emergence into plastic pots (6 cm diameter x 25 cm deep) individually filled with 600 g (dry weight) soil.

Invasive grass species mycorrhizal responsiveness study

This study assessed growth and root colonization of invasive warm-season grass species (*D. annulatum*, *P. ciliare*, or *H. contortus*) each planted into soil collected from beneath native warm-season grasses in non-invaded areas, adjacent to the monoculture stands of each invasive. We assessed mycorrhizal responsiveness of each species by comparing growth in native soil to that of growth in steam-pasteurized soil, thereby eliminating soil microbes. We also included an

inoculation treatment (15 g of living native soil added to one-half of the steam-pasteurization pots) to establish native microbial communities. The living soil inoculum was added directly below the seedling roots during seedling transplantation. Therefore, this study consisted of 3 invasive warm-season grass species each planted into 3 soil treatments (native soil; steam-pasteurized native soil; steam-pasteurized soil with native soil inoculum) x 5 replications.

Native grass species mycorrhizal responsiveness and feedback study

Our native species feedback study examined soil alterations indirectly by assessing growth and AM root colonization of native warm-season grass species (*C. cucullata*, *D. californica, P. bicolor*, or *S. vulpiseta*) each planted into soil collected from monoculture stands of three species of invasive grasses ('away') (*D. annulatum*, *P. ciliare*, or *H. contortus*). As a control, plant biomass production was assessed in plants grown in soils collected beneath native warm-season grasses ('home') in adjacent, non-invaded areas. We assessed the use of native soil inoculum as a soil amendment with possible restoration implications by adding 15 g of fresh (living) native soil (collected beneath native grasses in adjacent non-invaded grassland) to each of the 'away' (non-steamed) soils. We assessed mycorrhizal responsiveness of each species by comparing growth in native and all three invasive species soil to that of growth in steam-pasteurized soil, thereby eliminating soil microbes including AM fungi. To half of the pots in the steam-pasteurization treatment, 15 g of fresh (living) native soil (collected beneath native grasses in adjacent non-invaded grassland) was added as inoculum to establish native microbial communities. The living soil inoculum was added directly below the seedling roots during seedling transplantation. Therefore, this study consisted of 4 native warm-season grass species each planted into 4 soil origins (soil collected beneath three invasive warm-season grass species and a non-invaded control) x 4 soil treatments (nonsterile field soil; nonsterile + inoculum; steamed; and steamed + inoculum) x 5 replications. Native soil inoculum was not added to the nonsterile native prairie soil, as the microbial communities were redundant.

For both studies, pots were arranged in a randomized complete block design in a greenhouse maintained at 20-25 C and watered every other day. After 14 weeks plants were harvested and roots were washed free of soil. Shoot and root biomass were oven-dried for 72 h at 60 C. Shoot, root, and total plant dry weights were determined after drying. Subsamples of roots were stained with trypan blue, and scored for intraradical AM colonization using the magnified gridline intersect method (McGonigle et al. 1990).

Statistical analysis

All statistical analyses were conducted using R statistical software (R Core Team 2012). We analyzed our data using ANOVA with a complete block design. Prior to analysis, variances were analyzed for homogeneity using the modified robust Brown-Forsythe test (lawstat package; Kimihiro et al. 2009) and were determined to be homogeneous. Total AM colonization is presented as no clear trends were observed following assessment of individual types of AM fungal structures (hyphae, vesicles, arbuscules, and coil structures). For simplification of data presentation, only total dry weights are presented, as shoot, root, and reproductive dry weights were each highly correlated with total dry weight. We analyzed root/shoot ratios but no clear trends were observed and these data are not shown.

Comparison between species was not a focus of this study as differences in growth form are evident between species and thus each species was analyzed separately. Analysis of plant biomass production was conducted using a two-way analysis of variance for soil source (plant species that soil was collected beneath) x soil treatment (nonsterile field soil; nonsterile + inoculum; steamed; and steamed + inoculum). Significant differences between treatments were determined using the Tukey HSD test at an alpha level of 0.05. Correlation analyses were used to examine the relationships between root colonization of AM fungi and total plant biomass production.

RESULTS

Soil nutrient data indicated soil from all sites contained relatively low plant-available P and N and are typical of prairie soil from the Great Plains (Hartnett and Wilson 1999; Wilson et al. 2009; Johnson et al. 2010) (Table 2). Soil pH for all sites was in the neutral range (7.3-7.8), also typical of soils from prairie sites.

Invasive grass species mycorrhizal responsiveness study

In this study, we assessed the mycorrhizal responsiveness of three invasive (or encroaching) grass species of the southern Great Plains, USA by comparing biomass production in soils containing native soil microbial communities (field soil) with soil from which microbial communities were eliminated through steam-pasteurization. Two of the invasive grasses, *H. contortus* (native species that is encroaching) and *D. annulatum* (non-native invader) were highly responsive to AM fungi (obligate mycotrophs, requiring the symbiosis for growth) (Fig. 1a, b). However, *P. ciliare* (non-native invader) was non-responsive and did not require the symbiosis for growth (Fig. 1c). Inoculating steam-pasteurized native soils with native prairie soil resulted in similar biomass production as that of the native field soil; indicating steam-pasteurization of the soil did not induce adverse chemical or physical effects (Fig. 1a, b, c).

Plants grown in native soil that was not steamed were colonized by mycorrhizal fungi, as well as plants grown in steamed soil inoculated with nonsterile field soil. No roots from plants grown in steamed soil were colonized at experiment harvest (Fig. 1d, e, f). We found a significant correlation between production and mycorrhizal root colonization ($p < 0.001$; $R^2 = 0.527$) (Fig. 6) of the two mycorrhizal responsive species (*H. contortus* and *D. annulatum*). However, inclusion of the non-responsive species (*P. ciliare*) resulted in a large reduction in the relationship between biomass and colonization ($p < 0.001$; $R^2 = 0.185$; data not shown).

Native grass species mycorrhizal responsiveness and feedback study

In this study, we examined 1) the mycorrhizal dependency of the four native grass species to AM fungi; 2) the ability of native seedlings to grow in soil collected from areas dominated by native grasses ('home') compared to that of growth in soil collected from areas dominated by each of three invasive grass species ('away'); 3) the influence of native microbial communities added to each 'away' soil; and 4) the ability of native seedlings to grow in 'away' soils from which soil communities associated with the invasive were eliminated and native soil microbial communities were returned.

The mycorrhizal responsiveness of the four native species varied along a continuum from highly responsive to AM fungi (obligate mycotrophs, requiring the symbiosis for growth) to non-responsive (did not require the symbiosis for growth). Two of the native species (*P. bicolor* and *S. vulpisata*) did not grow in the absence of the symbiosis, regardless of soil source (home or away) (Fig. 2a-d; 3a-d). *Digitaria californica* biomass production was significantly less in steamed native soil, compared to the corresponding native ('home') field soil, yet was able to grow in the absence of soil microbial communities (Fig. 4a). Similarly, *D. californica* was able to grow in steamed 'away' soil (soil from an established plant community of each of the three invasive grasses), with reduced (Fig 4b) or similar biomass production (Fig 4c; 4d) as compared to growth in corresponding field soil. Steam-pasteurization of 'home' soil did not reduce biomass production of *Chloris cucullata*, compared to the corresponding native field soil (Fig. 5a). In fact, *C. cucullata* growth in steamed 'away' soil collected from sites dominated by *H. contortus*, *D. annulatum*, or *P. ciliare* was significantly greater than that of the corresponding field soil (Fig. 5b; c; d).

To assess the potential for native grasses to establish in soil collected beneath invasive grasses, native species were planted in freshly collected 'away' soil (soil from an established

plant community of each of the three invasive grasses). For comparison, native grass seedlings were planted into native prairie soil. Biomass production of two native species, *P. bicolor* and *C. cucullata*, was significantly reduced when grown in field soil collected from sites dominated by each of the three invasive grasses (*H. contortus*, *D. annulatum*, or *P. ciliare*) (Fig. 2a - d; Fig. 5a - d). Two of the native species, *S. vulpiseta* and *D. californica*, produced similar biomass when grown in soil from sites dominated by *H. contortus*, as compared to growth in native field soil (Fig. 3a; b; Fig. 4a; b). However, biomass of both of these native grasses was reduced when grown in soils collected from sites dominated by *D. annulatum* or *P. cilliare*, as compared to that of the native field soil (Fig. 3a; c; d; Fig. 4a; c; d).

To assess the restoration potential of native soil amendments to soils from areas occupied by invasive grasses, nonsterile field soil from native grassland areas were added to 'away' field soils prior to native seedling transplant. The addition of native field soil did not generally result in increased biomass production of any of the four native grasses (Fig. 2c; d; Fig. 3b-d; Fig. 4b-d; Fig. 5b-d). In fact, in only one species, *P. bicolor*, did addition of native field soil inoculum result in increased biomass production, compared to the corresponding field soil, and only with the native soil amendment to soil collected from the *H. contortus* dominated site (Fig. 2b).

Finally, we assessed the influence of native microbial communities added to steamed soil collected beneath invasive grasses (i.e. following elimination of soil microbial communities associated with the invasive grass, including AM fungi). Inoculating steamed native soils with native prairie soil resulted in similar biomass production as that of the native field soil; indicating steam-pasteurization of the soil did not induce adverse chemical or physical effects (Fig. 2a; 3a; 4a; 5a).

Addition of native soil inoculum to all three steamed 'away' soils show that the two native species *P. bicolor* and *S. vulpisata*, assessed as highly mycorrhizal responsive as in native

soils, and *D. californica*, the native species determined as facultatively responsive, resulted in biomass production equal or greater to that of the corresponding field soil, indicating the lack of growth in the steamed soil resulted from the elimination of native prairie microbial communities, including mycorrhizal fungi (Fig 2b-d; 3b-d; 4a-d). Inoculation of steamed 'away' soil with native field soil did not affect the biomass production of *C. cucullata*; growth in steamed soils and steamed with native soil inoculum were not significantly different in any of the 'away' soils (Fig. 5b-d).

All plants grown in 'home' or 'away' soil that was not steamed were colonized by mycorrhizal fungi, regardless of soil source. No roots from plants grown in steam-pasteurized soil were colonized at experiment harvest (Figs 1e-g; 2e-g; 3e-g; 4e-g). Biomass production of the native species was directly related to percent mycorrhizal root colonization, across all four native species we found a significant correlation between production and colonization ($p < 0.001$; $R^2 = 0.243$; data not shown). Furthermore, biomass production of mycorrhizal responsive species (*P. bicolor*, *S. vulpisata*, and *D. californica*) were tightly correlated with AM root colonization ($p < 0.001$; $R^2 = 0.529$) (Fig. 6).

DISCUSSION

Plants vary widely in their dependence on AM fungi; from highly dependent to parasitic depending on AM fungal species, plant host, and soil nutrient conditions (Johnson et al. 1997), and level of mycorrhizal dependency has been related to invasibility (Pringle et al. 2009; Vogelsang & Bever 2009). Therefore, as a first step, we assessed the mycorrhizal dependency of the invasive species in our study. Wilson and Hartnett (1998) examined 95 grassland plants and found a close relationship between host plant guilds, responsiveness, and AM root colonization, with perennial warm-season grasses being highly responsive to the symbiosis. Therefore, we hypothesized the invasive grasses of our study would be highly dependent on, or responsive to,

the symbiosis, as they are perennial warm-season grasses. In partial support of our hypothesis, two of our three invasive (or encroaching) species were highly responsive, with both *H. contortus* and *D. annulatum* requiring the symbiosis for biomass production and survival. However, *P. ciliare* was not responsive to AM symbiosis, producing similar biomass in steamed soil as the corresponding field soil. Interestingly, of 16 perennial grass species in the Wilson and Hartnett (1998) study, *Cynodon dactylon* [(L.) Pers.], an invasive species native to Africa, Asia, and Europe was substantially lower in AM responsiveness (similar to *P. ciliare*), compared to the native warm-season grasses assessed in their study.

Mycorrhizal dependence may play an important role in grassland invasion by non-native grasses, and these plant-fungal associations have been reported to both constrain (Vogelsang and Bever 2009) and facilitate (Shah et al. 2009) the ability of a non-native species to successfully invade. The growth and fitness of many invasive plant species have been found to be highly dependent on AM associations and studies indicate there can be a promotion in plant invasibility through their association with native AM fungi (Shah et al. 2008; Shah et al. 2009; Wilson et al. 2012). In contrast, Pringle et al. (2009) and Vogelsang & Bever (2009) provide strong evidence that many invasive plants are facultatively mycorrhizal, able to form associations with AM fungi, but not dependent on the symbiosis for survival. This alternative dependency may be a successful strategy of some invasive plant species. In fact, some non-native invasive species have evolved reduced dependence on AM fungi during the invasion of North America (Seifert et al. 2009), suggesting that there is a selective advantage to this strategy. Therefore, it is clear that invasive perennial warm-season grasses may respond differently to AM symbiosis, as compared to native grasses of this plant guild, and these species-specific responses may have restoration implications. We propose management practices for restoration may be most successful if determined on a species-specific and site-specific basis.

Because warm-season grasses have been shown to be highly mycotrophic, we hypothesized all four of the native warm-season perennial grasses in our study to be highly mycorrhizal responsive. In partial support of our hypothesis, both *P. bicolor* and *S. vulpisata* were obligately mycotrophic, producing little biomass when grown in steamed soil where AM fungi were eliminated. However, growth of *D. californica* and *C. cucullata* in steamed soil did not support our hypothesis. While growth of *D. californica* was significantly reduced in steamed soil, as compared to native field soil, this species was able to grow and produce biomass in the absence of the symbiosis, indicating this species is a facultative mycotroph, as opposed to obligately mycotrophic as we hypothesized. Furthermore, *C. cucullata* did not require the symbiosis; growth in steamed soils was equal or greater to that of the corresponding field soils. Because our steam-pasteurization eliminated the majority of native soil microbes, it is possible the increase in growth of *P. ciliare* in soil following pasteurization was due to removal of parasitic or pathogenic fungi from the soil (Torchin and Mitchell 2004). However, we did not see evidence of these fungi colonizing roots or damaging root tissue. The different mycotrophic dependencies of these native warm-season grasses maybe indicative of varying survival strategies; increasing competitive exclusion by increasing nutrient utilization and thereby decreasing invasion through limitation of non-native propagule establishment, effectively closing the "window of opportunity" for invasive species to enter the native community (Myster 1993; Davis et al. 2000; Agrawal et al. 2005).

However, invasive grass species of the southern and central Great Plains continue to expand their range. Data from our feedback study indicate that native species biomass production was consistently reduced when planted into 'away' soil, collected from beneath invasive warm-season grasses, as compared to 'home' field soil collected from adjacent prairie areas. When additions of live (non-sterile) soil collected from adjacent native prairie sites were added to steamed soil from the invaded sites, increased biomass production of native grasses was consistently observed. Across all soils, AM root colonization of the native grasses was strongly

correlated with biomass production, indicating that alterations in AM fungal communities may be contributing to the lack of growth in 'away' soils. This relationship was especially true for native grasses with a high responsiveness to the AM symbiosis, as has been reported in previous studies (Hetrick et al. 1990; Wilson & Hartnett 1998). However, due to the many soil microorganisms that can affect plant dynamics (Bever 2003), we cannot definitively attribute our increase in plant biomass production to any one group of organisms. Indeed, invasive plant species have been reported to alter both the composition and functional properties of rhizosphere biota (Weidenhamer and Calloway 2010). However, the close positive relationship between biomass production and AM root colonization is consistent with our hypothesis that mycorrhizae play an important role in the re-establishment of native prairie grasses, indicating the native AM fungal community may be a fundamental consideration to the successful establishment of native grasses into invaded sites.

Non-native plants have been shown to disrupt mycorrhizal mutualisms, giving them a competitive advantage over native plants (Harner et al. 2010; Jordan et al. 2012; Meinhardt and Gehring 2012). AM fungi can contribute to plant soil feedback, as AM fungal taxa can exhibit host-specific growth responses (Bever 2002), and the benefits a given plant receives can depend on the identity of its AM fungal associates (e.g. Johnson et al. 2010; Hoeksema et al. 2010). Non-native invasive plant species have been shown to alter the density and/or composition of the AM fungal communities and native plant root colonization, which may feedback on the subsequent spread of the introduced plant species (Barto et al. 2011; Grove et al. 2012; Meinhardt & Gehring 2012; Owen et al. 2013). Alternatively, invasive plants may benefit from AM fungi, by adapting to native fungi or altering the AM fungal community in a way that inhibits highly obligate native grassland species (Wilson et al. 2012). Host plants have been shown to shape distinctive AM fungal communities even when inoculated with the same AM fungal species and these altered communities can differentially impact growth of native and invasive plants (Bever et al. 1996;

Uibopuu et al. 2009). As plants can allocate preferentially to the most beneficial fungal partner (Kiers et al. 2011), it is possible that invasive plants may alter AM fungal communities to promote their own success. Moora et al. (2011) found non-native plants associated with non-host specific AM fungi, while the native plant-host species associated with a more diverse community of AM fungi, a change which may increase invasibility of non-natives. Interestingly, in our current study, both native species *S. vulpiseta* and *D. californica* produced significantly less biomass when grown in *D. annulatum* or *P. ciliare* soils, but biomass was not significantly reduced when grown in *H. contortus* soils. Both *D. annulatum* and *P. ciliare* are non-native invasive species, while *H. contortus* is a native encroaching species that is mycorrhizal dependent. It is possible that the lack of biomass reduction in these native grasses when grown in soil collected beneath *H. contortus* is because they evolved in concurrent ranges and associate with similar AM fungal communities (Ji et al. 2013). It is also possible that the shared evolutionary history of these native species allows occupation of separate niches, effectively reducing competitive overlap.

While our results support our hypothesis that invasive grass feedback occurs through alterations in biotic communities, suppression of native plant growth appear to include other mechanisms, as well. An alternative or additive mechanism could be chemical alterations in which invasive species release exudates that directly kill native species or prevents native species from germinating or establishing (Grove et al. 2012; Hu and Zhang 2013). Inderjit and van der Putten (2010) theorize that native plant species associate with soil microbes that have evolved with native plant-produced compounds, resulting in the selection of microbial species capable of degrading these compounds. If native soil microbes are exposed to novel chemicals they have not evolved with, such as those produced by non-native plant species, their inability to degrade these chemicals could result in accumulation to toxic levels. If the allelopathic chemicals produced by the non-native are not able to be degraded by native microbes, sterilizing and re-inoculating with

native microbial communities would not be expected to result in a positive growth response by our native grasses. In our current study, soil steam-pasteurization and re-inoculation with native soil microbial communities frequently resulted in an increase in plant biomass production, such as was observed by the corresponding native field soil (e.g. *P. bicolor* in *H. contortus* soil or *S. vulpisata* in *H. contortus* or *P. ciliare* soil). However, several of our native grasses did not achieve similar biomass production following steam-pasteurization and re-inoculation with native prairie soil (e.g. *P. bicolor* in *D. annulatum* or *P. ciliare* soil or *D. californica* in *D. annulatum* soil). Therefore, while our results indicate plant growth suppression following invasion by these non-natives is, at least partially, through the alteration in soil microbial communities, our data also suggests other mechanisms may be involved in plant-soil feedback resulting in non-native plant species success. Soil from monocultures of *D. annulatum*, an invasive species of our current study, was highly suppressive to growth of each of the native species tested in our study. Greer (2013) found that *Bothriochloa ischaemum* [(L.) Keng), a non-native species belonging to the same group of perennial warm-season invasive grasses as *D. annulatum* (referred to as old world bluestems), released allelopathic chemicals that effectively eliminated germination and establishment of native warm-season grasses, with no conspecific effects. Dirvi and Hussain (1979) have shown allelopathic effects of *D. annulatum* on some species of cultivated crops in Pakistan, an area where *D. annulatum* is native.

Invasive species have also been shown to release chemical exudates that disrupt symbiotic relationships with soil microbes, directly inhibiting the ability of AM fungi to colonize these native grasses, or indirectly reducing AM fungal colonization by suppressing the growth of the native grasses, thereby reducing carbon allocations to the symbiont (Roberts and Anderson 2001; Stinson et al. 2006; Abhilasha et al. 2008; Callaway et al. 2008; Inderjit & van der Putten 2010; Grove et al. 2012). We are not able to determine if the non-native invasive plant species altered the composition of the AM fungal communities in our current study, as we did not

conduct molecular analysis of roots and the surrounding soil. These analyses would provide valuable insight in determining if the AM fungal communities associating with the invasive grasses differed from those associating with native species. While molecular analysis was beyond the scope of this study, M. Greer (personal communication, Oklahoma State University) is conducting these assessments through analyses of field-collected roots and soils for several of the invasive and native warm-season grasses of our current study.

Plant species alter soil conditions and these alterations can remain as soil carry-over-effects, or soil legacies (Bartelt-Ryser et al. 2005) and these effects can persist in the soil for varying periods of time after the plant species has been removed (van der Putten et al. 2013). If these invasive grasses have created soil legacies, then mere removal may not be sufficient for successful restoration of native plant communities (van der Putten et al. 2013). Restoration success following removal of invasive plants may be improved through inoculation with native AM fungi. However, in our current study, plant biomass production was not significantly improved in non-steamed 'away' soils following re-inoculation with native soil, as compared to growth in soil collected directly beneath invasive plants. Dry weight of plants inoculated with native prairie soil and grown in soil collected from monocultures of invasive grasses did not achieve the growth of plants grown in soils collected from the adjacent native prairie field soil. It is possible legacy effects of the invasive plants were not completely ameliorated in this 14 week study. Re-inoculation of the native soil microbial communities was accomplished by the addition of 15 g of native soil into 600 g sterilized field soil. It is likely the soil microbial population densities of the re-inoculated treatment did not reach that of the native, non-sterilized soil treatment in our 14 week study. It is also possible that other mechanisms, such as production of allelopathic chemicals, were not alleviated by the addition of native soil inoculum. In a greenhouse study assessing the restoration potential of native AM fungal inoculation following invasion by *Bothriochloa spp.*, Wilson et al. (2012) reported significant increases in native plant

species production following re-inoculation with native soil, but the adverse effects of the *Bothriochloa spp.* were not completely ameliorated in their 14 week study. However, inoculation with native AM fungi has been demonstrated as a potential restoration tool in several field studies. For example, the degradation of the AM fungal community by invasive grasses was alleviated through restoration efforts involving inoculation with native AM fungi, resulting in increases in growth and survival of prairie plants, with later successional plants being the most responsive (Middleton and Bever 2012). Similarly, Rowe et al. (2009) found that adding soil from intact plant communities containing native AM fungal propagules reduced cheatgrass cover and increased native perennial cover. Further studies, especially those involving field studies, will be essential for providing information concerning successful restoration of invaded sites following inoculation with native field soils.

Invasion of native grasslands by non-native grass species has altered composition of soil, plants, and wildlife, as well as imposing negative effects on domestic livestock production and loss of ecosystem function. Eradication of non-native invasive species and subsequent reestablishment of native microbes and grass species is imperative to returning ecosystem function. The results from our current greenhouse study indicate that a species-by-species restoration plan may be most successful. Both native and invasive warm-season grasses varied in mycorrhizal dependency, and production of the four native species varied in response to each invasive grass, and in response to native soil inoculum additions. A key goal in restoration following eradication of non-native monocultures is the establishment of native species, as establishment of native species has been shown been shown to decrease establishment of invasive species following restoration (Middleton et al. 2009). Understanding how soil alterations, including microbial associations and production of allelopathic compounds, influence plant invasions may be a critical aspect of the restoration and conservation of native grassland ecosystems.

REFERENCES

Abhilasha D, Quintana N, Vivanco J, Joshi J (2008) Do alleopathic compounds in invasive Solidago canadensis s.l. restrain the native European flora? J Ecol 96:993-1001

Albrecht SL, Okon Y, Lonnquist J, Burton RH (1981) Nitrogen fixation by corn-*Azospirillium* associations in a temperate climate. Crop Sci 21:301-306

Agrawal AA, Kotanen PM, Mitchell CE, Power AG, Godsoe W, Kilronomos J (2005) Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology 86:2979-2989

Augspurger CK (1990) Spatial patterns of damping-off disease during seedling recruitment in tropical forests. In: Burdon JJ, Leather SR (eds) Pest, Pathogens and Plant Communities. Blackwell Scientific Publications, Oxford, UK, pp 131-144

Bartelt-Ryser J, Joshi J, Schmid B, Brandl H, Balser T (2005) Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspect Plant Ecol 7:27-49

Barto EK, Antunes PM, Stinson K, Koch AM, Klironomos JN, Cipollini D (2011) Differences in arbuscular mycorrhizal fungal communities associated with sugar maple seedlings in and outside of invaded garlic mustard forest patches. Biol Invasions 13: 2755-2762

Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two invasive plants alter soil microbial community composition in serpentine grasslands. Biol Invasions 8:217-230

Bever JD (2002) Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. P R Soc London. 269:2595-2601

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical test. New Phytol 157:465-473

Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M (2010) Rooting theories of plant community ecology in microbial interactions. Trends Ecol Evol 25:468-478

Bever JD, Morton JB, Antonvics J, Schultz PA (1996) Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J Ecol 84:71-82

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: The utility of the feedback approach. Ecology 85:561-573

Callaway RM, Cipollini D, Barto K, Thelen GC, Hallett SG, Prati D, Stinson K, Kilronomos J (2008) Novel weapons: invasive plant supresses fungal mutualist in America but not in its native Europe. Ecology 89:1043-1055

Chapin FS III, Zavaleta ES, Eviner VT, Naylor RT, Vitousek PM, Reynolds HL, Hooper DU (2000) Consequences of changing biodiversity. Nature 405:234-242

D'Antonio CM, Vitousek PM (1992) Biological invasion by exotic grasses, the grass-fire cycle, and global change. Annu Rev of Ecol Syst 23:63-87

Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: A general theory of invasibility. J Ecol 88:528-534

Davis MA, Grime JP, Thompson K (2001) Charles S. Elton and the dissociation of invasion ecology from the rest of ecology. Divers Distrib 7:97-102

Denison RF (2000) Legume and sanctions and the evolution of symbiotic cooperation by *Rhisobia*. Am Nat 156:567-576

DerylocM, Skorupska A (1992) Rhizobial siderophore as an iron source for clover. Physiol Plantarum 85:549-553

Dirvi GA, Hussain F (1979) Allelopathic effects of *Dichanthium annulatum* (Forsk) Stapf on some cultivated plants. Pakistan J Sci Ind R 22:194-197

Fulbright TE, Hickman KR, Hewitt DG (2013) Exotic grass invasion and wildlife and wildlife abundance and diversity, south-central United States. Wildlife Soc B 37:503-509

Gonzalez L, DallaRosa J (2006) The Quiet Invasion: A Guide to Invasive Plants of the Galveston Bay Area. Houston Advanced Research Center, Houston, TX

Gould FW (1975) Texas plants: a checklist and ecological summary. Texas Agricultural experiment station, College Station, TX. USA.

Greer MJ (2013) Grassland invasion by non-native grass species: Ecological issues for multiple species at multiple trophic levels. Dissertation, Oklahoma State University

Griffin, GF (1993) The spread of buffelgrass in inland Australia: Land use conflicts. In: Wilson BJ, Swarbick JT (eds.) Proceedings of the 10th Australian Weeds Conference and 14th Asian Pacific Weed Science Society Conference. Brisbane, Queensland. 6 - 10 September 1993. The Weed Society of Queensland, Brisbane, QLD, pp.501-504

Grove S, Haubensak KA, Parker IM (2012) Direct and indirect effects of allelopathy in the soil legacy of an exotic plant invasion. Plant Ecol 213:1869-1882

Harmoney KR, Hickman KR (2004) Comparing morphology of caucasian old world bluestem and native grass. Agron J 96:1540-1544

Harmoney KR, Stahlman PW, Hickman KR (2007) Supression of caucasian old world bluestem with split application of herbicides. Weed Technol 21:573-577

Harner MJ, Mummey DL, Stanford JA, Rillig MC (2010) Arbuscular mycorrhizal fungi enhance spotted knapweed growth across a riparian chronosequence. Biol Invasions 12:1481-1490

Hartnett DC, Wilson GWT (1999) Mycorrhizal mediation of plant species composition and diversity in tallgrass prairie. Ecology 80:122-130

Hetrick BAD, Wilson GWT, Todd TC (1990) Differential responses of C3 and C4 grasses to mycorrhizal symbiosis, P fertilization, and soil microorganisms. Can J Botany 68:461-467

Heywood VH (1989) Patterns, extents and modes of invasions by terrestrial plants. In: Drake JA, Mooney HA, di Castri F, Grovers RH, Kruger FJ, Rejmanek M, Williamson M (eds) Biological invasions: a global perspective. Wiley, Chichester, UK, pp 31-60

Hickman KR, Farley GH, Channell R, Steier JE (2006) Effects of old world bluestem (*Bothriochloa ischaemum*) on food availability and avian community composition within the mixed-grass prairie. Southwest Nat 51:524-530

Hoeksema JD, Chaudhary VB, Gehring CS, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GWT, Klironomos JN, Umbanhowar J (2010) Context-dependency in plant response to mycorrhizal fungi: A meta-analysis. Ecol Lett 13:394-407

Hu G, Zhang ZH (2013) Allelopathic effects of *Chromolaena odorata* on native and non-native herbs. J Food Agric Environ 11:878-882.

Inderjit, van der Putten WH (2010) Impacts of soil communities on exotic plant invasions. Trends Ecol Evol 25:512-519

Ji BM, Gehring CA, Wilson GWT, Miller RM, Flores-Renteria L, Johnson NC (2013) Patterns of diversity and adaptation in *Glomeromycota* from three prairie grasses. Mol Ecol 22:2573-2587

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 135:575-585

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation as a driver of local adaptation in mycorrhizal symbioses. P Natl Acad Sci USA 107:2093-2098

Jordan NR, Aldrich-Wolfe L, Huerd SC, Larson DL, Muehlbauer G (2012) Soil-occupancy effects of invasive and native grassland plant species on composition and diversity of mycorrhizal associations. Invasive Plant Sci Manag 5:494-505

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoorhuyse P, Jansa J, Bucking H (2011) Reciporical rewards stabilize cooperation in the Mycorrhizal Symbiosis. Science 333: 880-882

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion ecology. Blackwell Publishing, Malden, MA

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol 115:495-501

Meinhardt KA, Gehring CA (2012) Disrupting mycorrhizal mutualisms: A potential mechanism by which exotic tamarisk outcompetes native cottonwoods. Ecol Appl 22:532-549

Middleton EL, Bever JD (2012) Inoculation with a native soil community advances succession in a grassland restoration. Restor Ecol 20:218-226

Middleton E L, Bever JD, Schultz PA (2009) The effect of restoration methods on the quality of the restoration and resistance to invasion by exotics. Restor Ecol 18:181-187

Moora M, Berger S, Davison J, Opik M, Bommarco R, Bruelheide H, Kuhn I, Kunin WE, Metsis M, Rortais A, Vanatoa A, Vanatoa E, Stout JC, Truusa M, Westphal C, Zobel M, Walther GR (2011) Alien plants associate with widespread generalist arbuscular mycorrhizal fungal taxa: Evidence from a continental-scale study using massively parallel 454 sequencing. Journal Biogeogr 38:1305-1317

Myster RW (1993) Tree invasion and establishment in old fields at Hutcheson Memorial Forest. Bot Rev 59:251-572

Noguchi K, Hui WLW, Gel YR, Gastwirth JL, Miao W (2009) lawstat: An R package for biostatistics, public policy, and law. R package version 2.3

Norwine J, Harriss R, Yu J, Tebaldi C, Bingham R (2007) The problematic climate of Texas 1900-2100. In: Norwine J, John K (eds) The changing climate of south Texas 1900–2100: Problems and prospects, impacts and implications. Book masters, Ashland, OH, pp. 15-57

Orr SP, Rudgersm JA, Clay K (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. Plant Ecol 181:153-165

Owen SM, Sieg CH, Johnson NC, Gehring CA (2013) Exotic cheatgrass and loss of soil biota decrease the performance of a native grass. Biol Invasions 15:2503-2517

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN (2009) Mycorrhizal symbioses and plant invasions. Annu Rev Ecol Evol S 40:699-715

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reinhart KO, Calloway RM (2006) Soil Biota and invasive plants. New Phytol 170: 445-457

Reinhart KO, Packer A, Van der Putten WH, Clay K (2003) Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. Ecol Lett 6:1046-1050

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grass roots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84:2281-2291

Roberts KJ, Anderson RG (2001) Effects of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. Am Mid Nat 146:146-152

Rowe HI, Brown CS, Paschke MW (2009) The influence of soil inoculum and nitrogen availability on restoration of high-elevation steppe communities invaded by *Bromus tectorum.* Restor Ecol 17:686-694

Seifert EK, Bever JD, Maron JL (2009) Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. Ecology 90:1055-1062

Shah MA, Reshi ZA, Khasa D (2009) Arbuscular mycorrhizal status of some Kashmir Himalayan alien invasive plants. Mycorrhiza 20:67-72

Shah MA, Reshi ZA, Reshi I (2008) Mycorrhizosphere mediated mayweed chamomile invasion in the Kashmir Himalaya, India. Plant Soil 312:219-225

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D, Kilronomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. Plos Bio Biology 4:727-731

Tognetti PM, Chaneton EJ (2012) Invasive exotic grasses and seed arrival limit native species establishment in an old-field grassland succession. Biol Invasions 14:2531-2544

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. Front Ecol Environ 2:183-190

Uibopuu A, Moora M, Saks U, Daniell T, Zobel M, Opik M (2009) Differential effect of arbuscular mycorrhizal fungal communities from ecosystems along management gradient on the growth of forest understory plant species. Soil Biol Biochem 41:2141-2146

USDA NRCS (U.S. Department of Agriculture, Natural Resource Conservation Service) (2012) The PLANTS database: National Plant Data Team, Greensboro, NC. http://plants.usda.gov. Accessed 5 June 2012

Van der Patten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, Van der Voorde TFJ, Wardle DA (2013) Plant-soil feedbacks: the past, the present, and future challenges. J Ecol 101:265-276

Van der Putten WH, Kowalchuk GA, Brinkman EP, Doodeman GTA, van der Kaaji RM, Kamp AFD, Menting FBJ, Veenedaal EM (2007) Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity. Ecology 88:978-988

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. Science 277:494-499

Vitousek PM, Walker LR (1989) Biological invasions by Myrica-faya in Hawaii-plant demography, nitrogen-fixation, ecosystem effects. Ecol Monogr 59:247-265

Vogelsang KM, Bever JD (2009) Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology 90:399-407

Watkinson AR, Ormerod SJ (2001) Grasslands, grazing and biodiversity: Editors' introduction. J Appl Ecol 38:233-237

Weidenhamer JD, Calloway RM (2010) Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. J Chem Ecol 36:59-69

Westover KM, Bever JD (2001) Mechanisms of plant species coexistence: Roles of rhizosphere bacteria and root fungal pathogens. Ecology 82:3285-3294

Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in prairie grasses and forbs. Am J Bot 85:1732-1738

Wilson GWT, Hickman KR, Williamson MM (2012) Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. Mycorrhiza 22:327-336

Wilson, GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. Ecol Lett 12:452-46

TABLES

Table 1. Non-native and native warm-season grass species utilized in this study. Listed by scientific and common name, species status in the U.S, species origin, states where species is currently problematic, and purpose of species introduction.

Scientific Name	Common Name	Status	Origin	States with Issues	Purpose of Introduction
Heteropogon contortus[1]	Tanglehead	Native/Encroaching	North America	TX, AZ, FL, HI, NM, CA	N/A
Dichanthium annulatum[2]	Kleberg Bluestem	Non-native/Invasive	Asia, Africa	TX, LA, HI	Forage and Erosion Control
Pennisetum ciliare[3]	Buffelgrass	Non-native/Invasive	Asia, Africa, Europe	TX, AZ, CA, FL HI, LA, MO, NM, NY, OK	Forage
Chloris cucullata	Hooded Windmillgrass	Native	North America	N/A	N/A
Digitaria californica	Arizona Cottontop	Native	North America	N/A	N/A
Pappophorum bicolor	Pink Pappusgrass	Native	North America	N/A	N/A
Seteria vulpiseta	Plains Bristlegrass	Native	North America	N/A	N/A

1 – USDA 2012.

2 – Gonzalez and DallaRosa 2006.

3 – Griffin 1993.

Table 2. Plant-available N and P, and pH of soil collected from native prairie or monocultures of encroaching (*Heteropogon contortus*) or non-native (*Dichanthium annulatum*; *Pennisetum ciliare*) warm-season grasses.

Dominant plant species	$P\text{-}PO_4$(mg kg^{-1})	$N\text{-}NH_4$ (mg kg^{-1})	$N\text{-}NO_3$ (mg kg^{-1})	pH
Native Prairie	22.5	6.93	3.50	7.6
H. contortus	16.5	8.03	5.00	7.3
P. ciliare	12.0	11.36	6.00	7.7
D. annulatum	8.5	8.45	1.00	7.8

FIGURES

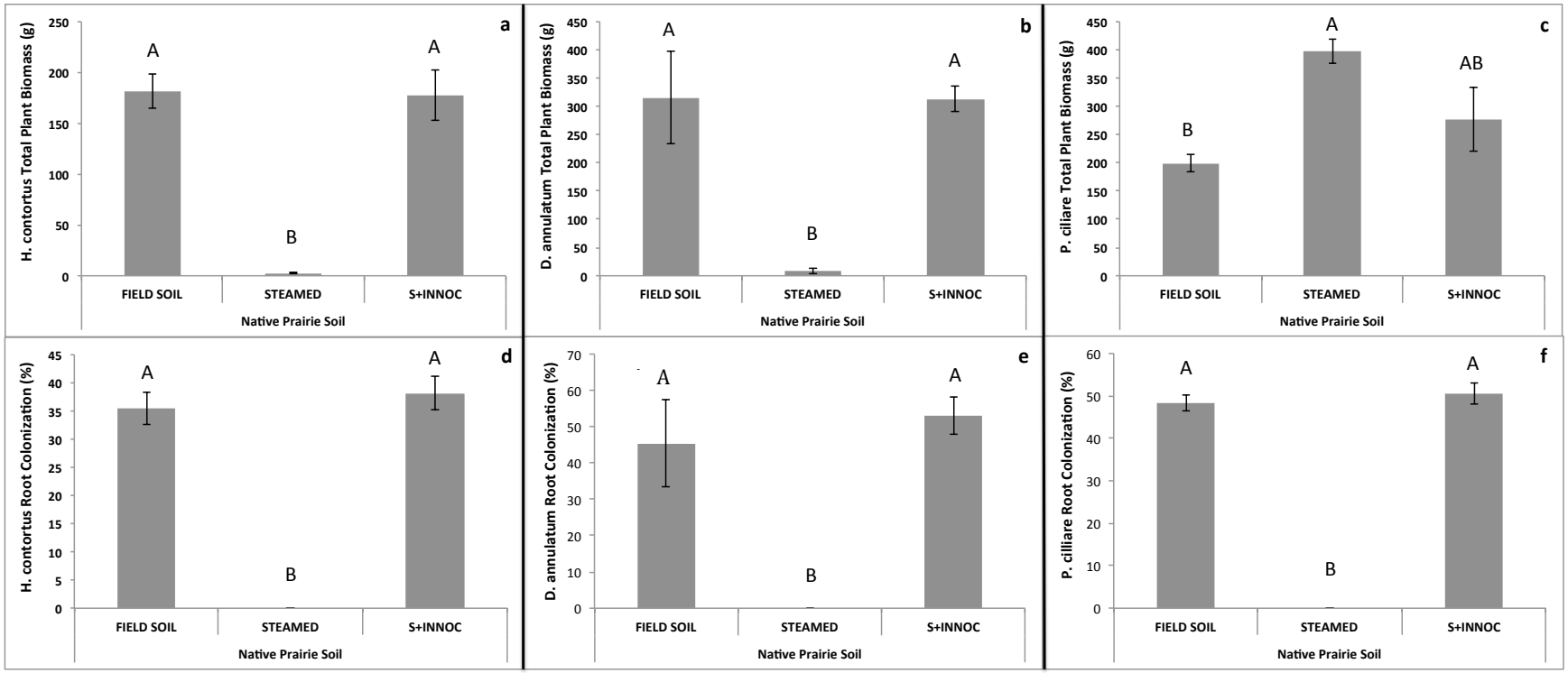


Figure 1.

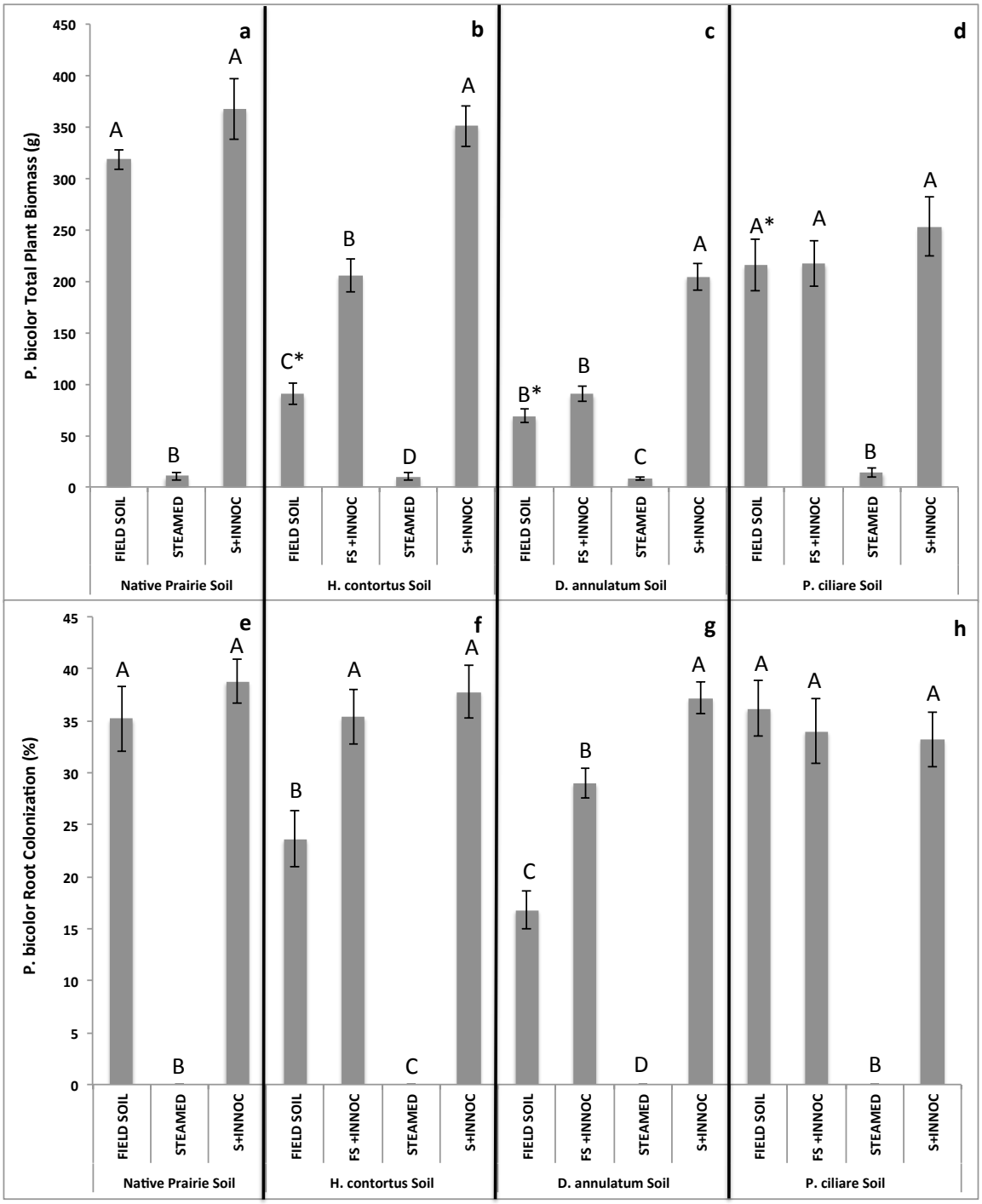

a
b
c
d
e
f
g
h
P. bicolor Total Plant Biomass (g)
P. bicolor Root Colonization (%)
FIELD SOIL
STEAMED
S+INNOC
FS +INNOC
Native Prairie Soil
H. contortus Soil
D. annulatum Soil
P. ciliare Soil

Figure 2.

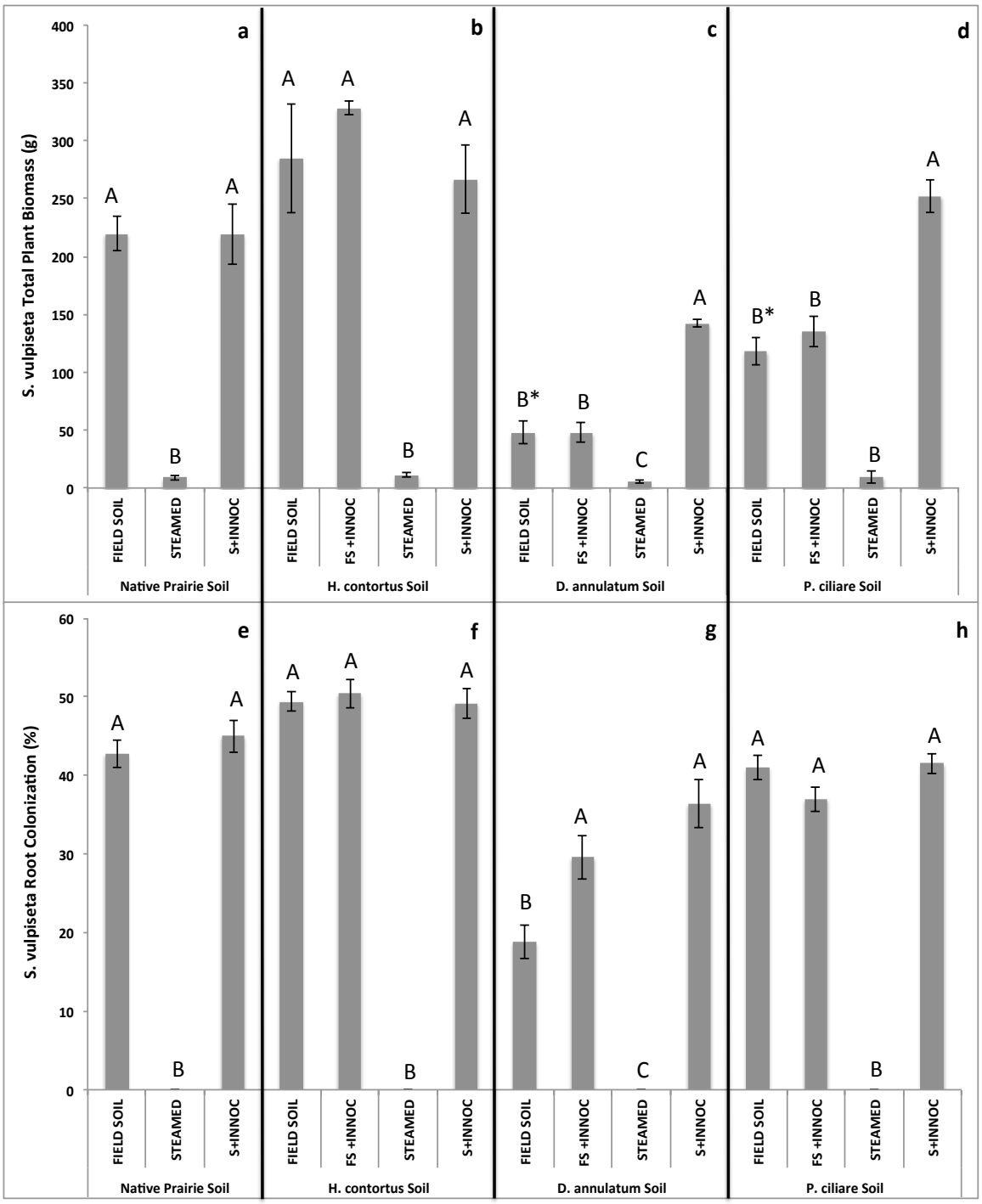

a
b
c
d
e
f
g
h
S. vulpiseta Total Plant Biomass (g)
S. vulpiseta Root Colonization (%)
FIELD SOIL
STEAMED
S+INNOC
FS +INNOC
Native Prairie Soil
H. contortus Soil
D. annulatum Soil
P. ciliare Soil

Figure 3.

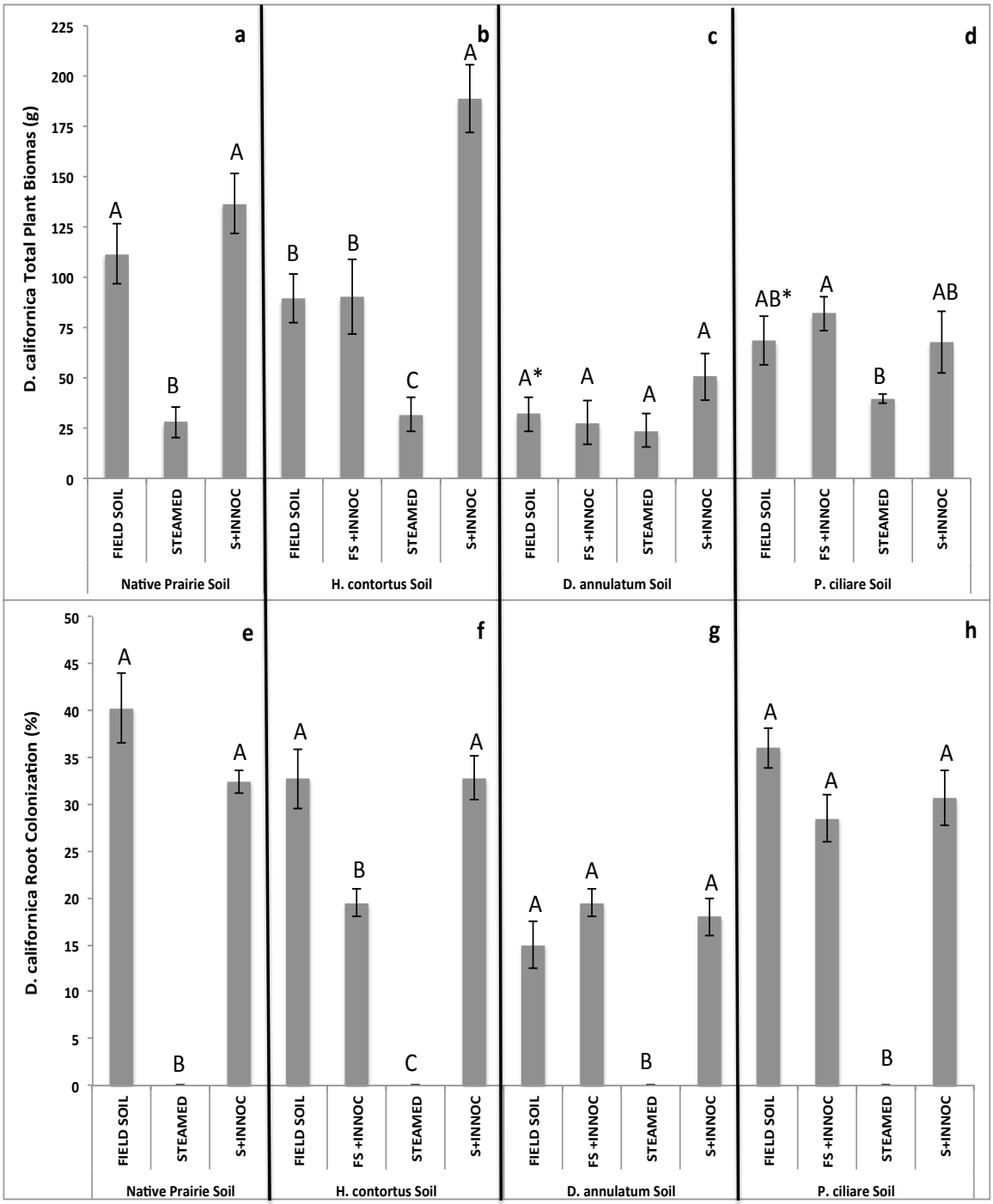

a
b
c
d
e
f
g
h
D. californica Total Plant Biomas (g)
D. californica Root Colonization (%)
FIELD SOIL
STEAMED
S+INNOC
FS +INNOC
Native Prairie Soil
H. contortus Soil
D. annulatum Soil
P. ciliare Soil

Figure 4.

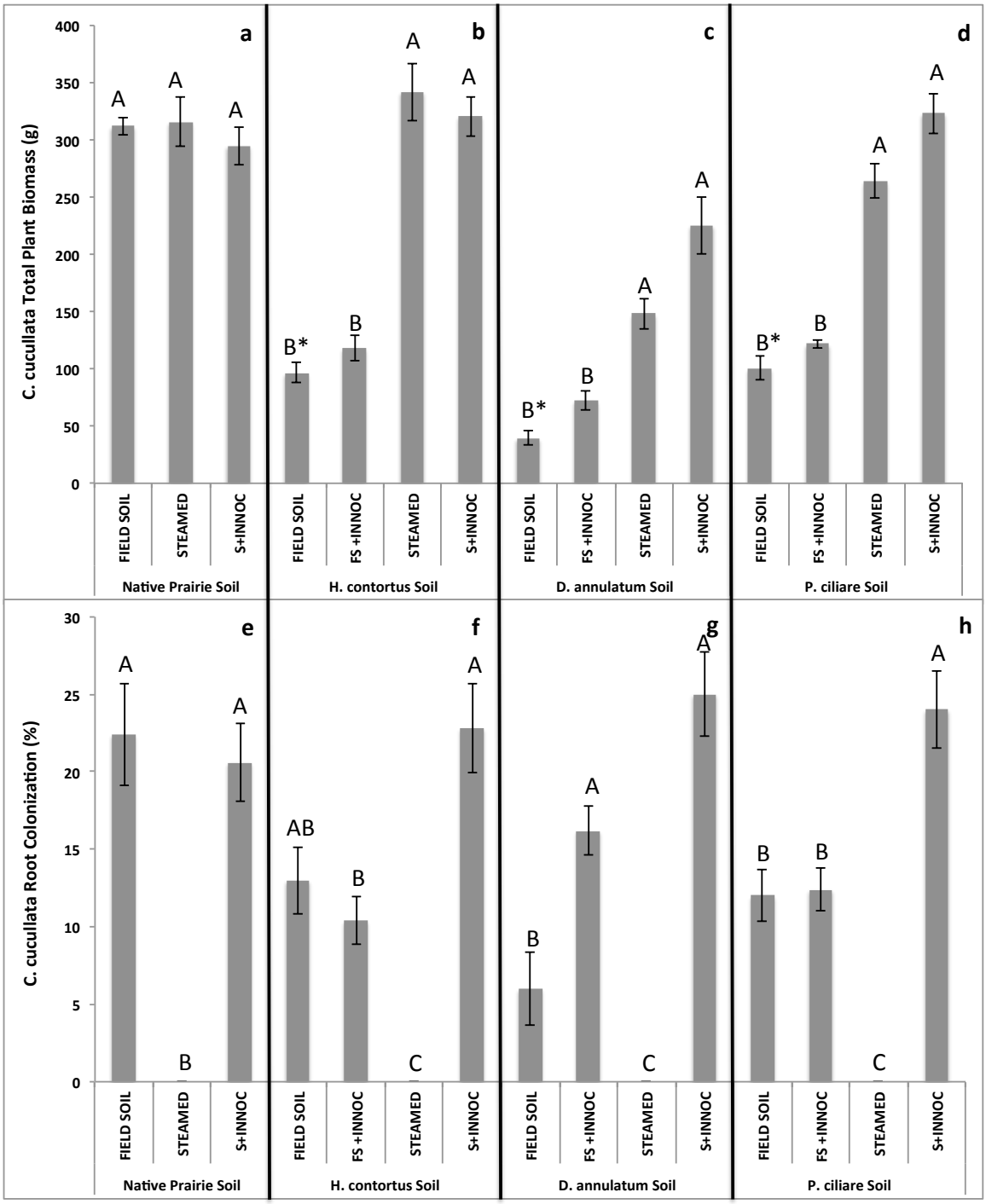

C. cucullata Total Plant Biomass (g)
C. cucullata Root Colonization (%)
a
b
c
d
e
f
g
h
FIELD SOIL
STEAMED
S+INNOC
FS +INNOC
Native Prairie Soil
H. contortus Soil
D. annulatum Soil
P. ciliare Soil

Figure 5.

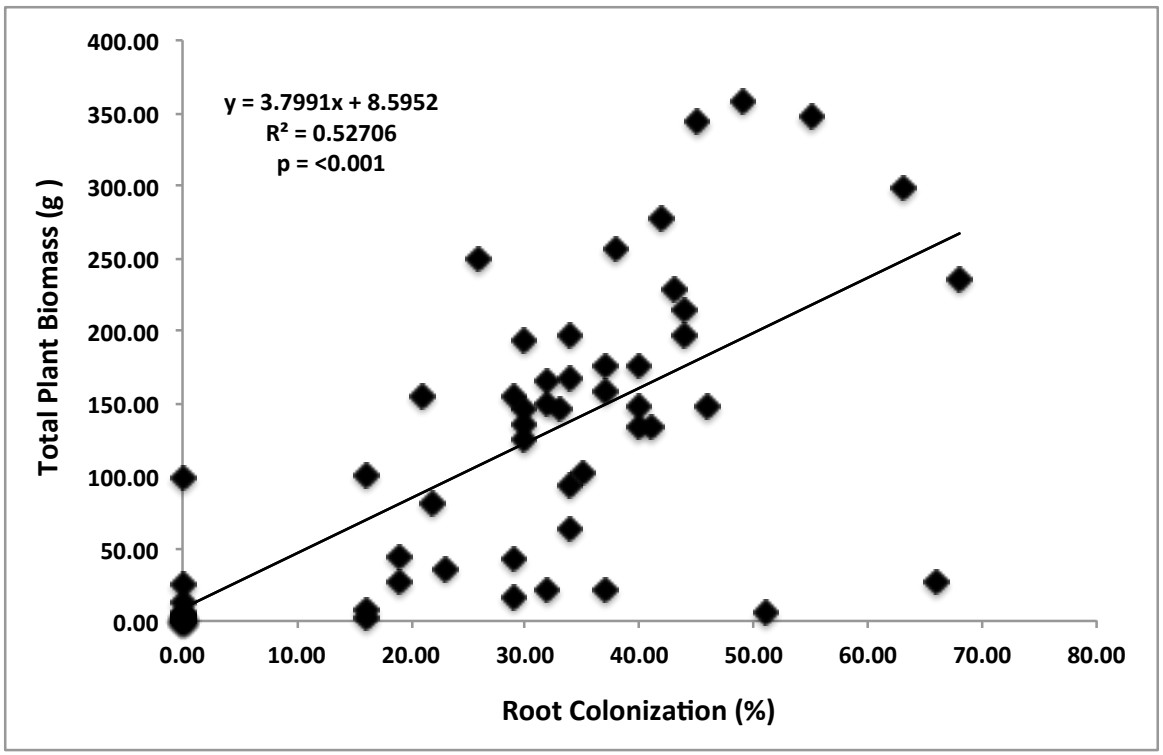

y = 3.7991x + 8.5952
R² = 0.52706
p = <0.001
Total Plant Biomass (g)
Root Colonization (%)
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00

Figure 6.

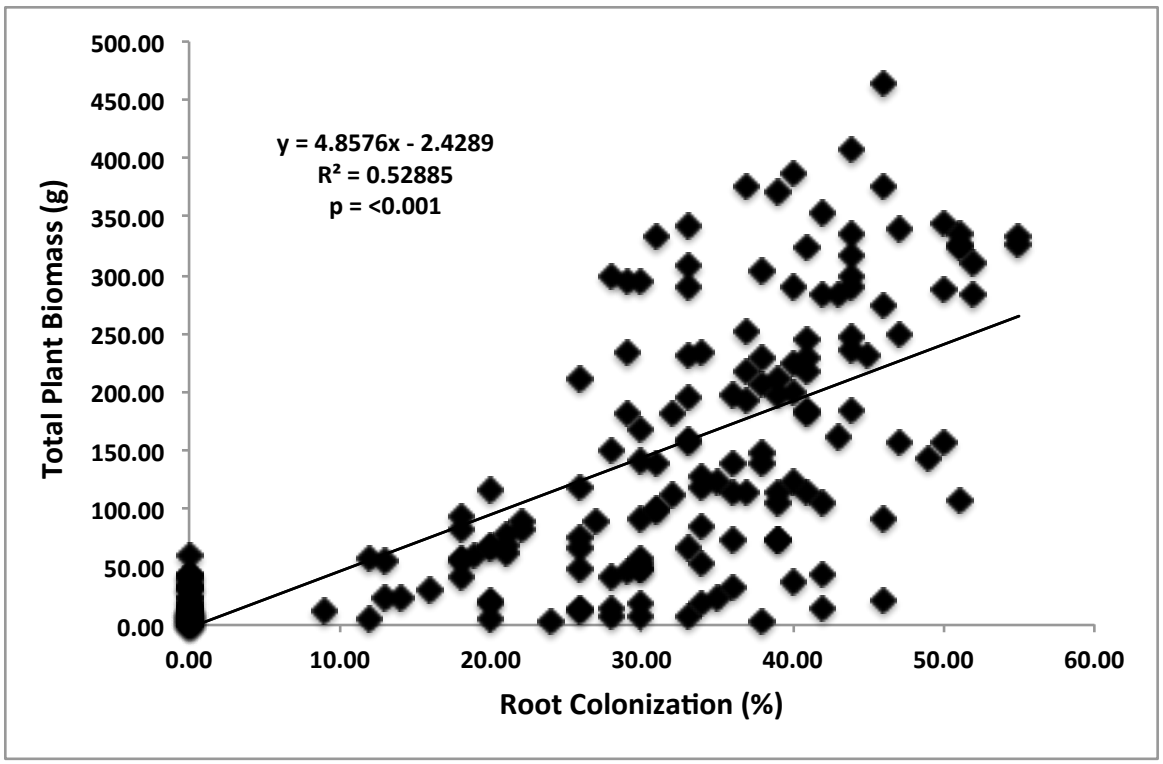

500.00
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Total Plant Biomass (g)
y = 4.8576x - 2.4289
R² = 0.52885
p = <0.001
0.00
10.00
20.00
30.00
40.00
50.00
60.00
Root Colonization (%)

Figure 7.

FIGURE CAPTIONS

Figure 1: Total plant dry weight (shoot plus root) and percent arbuscular mycorrhizal fungal root colonization of (a, d) *Heteropogon contortus* (Tanglehead, encroaching); (b, e) *Dichanthium annulatum* (Kleberg bluestem, invasive); and (c, f) *Pennisetum ciliare* (Buffelgrass, invasive) grown in soils collected from native prairie. Soils were: left untreated (FIELD SOIL); steamed to remove native microbial communities including arbuscular mycorrhizal fungi (STEAMED), or steamed and inoculated with native soil (S+INNOC). Error bars show $\pm$ 1 standard error. Bars with the same letter are not significantly different ($P \geq 0.05$).

Figure 2: Total plant dry weight (shoot plus root) and percent arbuscular mycorrhizal fungal root colonization of *Pappophorum bicolor* (Pink Pappusgrass, native) grown in native grassland soil (a, e); soil collected from monoculture of *Heteropogon contortus* (b, f); soil collected from monoculture of *Dichanthium annulatum* (c, g), or soil collected from monoculture of *Pennisetum ciliare* (d, h). Soils from each collection site were left untreated (FIELD SOIL), steamed to remove native microbial communities including arbuscular mycorrhizal fungi (STEAMED), left untreated and inoculated with native soil (FS+INNOC), or steamed and inoculated with native soil (S+INNOC). Error bars show $\pm$ 1 standard error. Bars with the same letter are not significantly different ($P \geq 0.05$). "*" indicates biomass of plants grow in 'away' field soil was significantly different compared to biomass production of plants grown in 'home' field soil.

Figure 3: Total plant dry weight (shoot plus root) and percent arbuscular mycorrhizal fungal root colonization of *Setaria vulpiseta* (Plains Bristlegrass, native) grown in native grassland soil (a, e); soil collected from monoculture of *Heteropogon contortus* (b, f); soil collected from monoculture of *Dichanthium annulatum* (c, g), or soil collected from monoculture of *Pennisetum ciliare* (d, h). Soils from each collection site were left untreated (FIELD SOIL), steamed to remove native microbial communities including arbuscular mycorrhizal fungi (STEAMED), left untreated and

inoculated with native soil (FS+INNOC), or steamed and inoculated with native soil (S+INNOC). Error bars show ± 1 standard error. Bars with the same letter are not significantly different ($P \geq 0.05$). "*" indicates biomass of plants grow in 'away' field soil was significantly different compared to biomass production of plants grown in 'home' field soil.

Figure 4: Total plant dry weight (shoot plus root) and percent arbuscular mycorrhizal fungal root colonization of *Digitaria californica* (Arizona Cottontop, native) grown in native grassland soil (a, e); soil collected from monoculture of *Heteropogon contortus* (b, f); soil collected from monoculture of *Dichanthium annulatum* (c, g), or soil collected from monoculture of *Pennisetum ciliare* (d, h). Soils from each collection site were left untreated (FIELD SOIL), steamed to remove native microbial communities including arbuscular mycorrhizal fungi (STEAMED), left untreated and inoculated with native soil (FS+INNOC), or steamed and inoculated with native soil (S+INNOC). Error bars show ± 1 standard error. Bars with the same letter are not significantly different ($P \geq 0.05$). "*" indicates biomass of plants grow in 'away' field soil was significantly different compared to biomass production of plants grown in 'home' field soil.

Figure 5: Total plant dry weight (shoot plus root) and percent arbuscular mycorrhizal fungal root colonization of *Chloris cucullata* (Hooded Windmillgrass, native) grown in native grassland soil (a, e); soil collected from monoculture of *Heteropogon contortus* (b, f); soil collected from monoculture of *Dichanthium annulatum* (c, g), or soil collected from monoculture of *Pennisetum ciliare* (d, h). Soils from each collection site were left untreated (FIELD SOIL), steamed to remove native microbial communities including arbuscular mycorrhizal fungi (STEAMED), left untreated and inoculated with native soil (FS+INNOC), or steamed and inoculated with native soil (S+INNOC). Error bars show ± 1 standard error. Bars with the same letter are not significantly different ($P \geq 0.05$). "*" indicates biomass of plants grow in 'away' field soil was significantly different compared to biomass production of plants grown in 'home' field soil.

Figure 6: The relationship between arbuscular mycorrhizal root colonization and total plant biomass (shoot plus root) of the two AM fungal responsive (obligate and facultative mycotrophs) invasive grass species (*Heteropogon contortus* and *Dichanthium annulatum*) (N=70) grown in soils collected from native prairie and areas with established monocultures of invasive grasses (*Heteropogon contortus*, *Dichanthium annulatum*, or *Pennisetum ciliare*.

Figure 7: The relationship between arbuscular mycorrhizal root colonization and total plant biomass (shoot plus root) of the three AMF responsive (obligate and facultative mycotrophs) native grassland species (*Pappophorum bicolor*, *Setaria vulpiseta*, and *Digitaria californica*) (N=225) grown in soils collected from native prairie and areas with established monocultures of invasive grasses (*Heteropogon contortus*, *Dichanthium annulatum*, or *Pennisetum ciliare*.

CHAPTER II

A STORY OF CHEMICAL WARFARE IN NATURE: ARE INVASIVE WARM-SEASON GRASSES PRODUCING ALLELOPATHIC BIOCHEMICALS TO INVADE GRASSLANDS OF THE CENTRAL AND SOUTHERN GREAT PLAINS

ASTRACT

Background and Aims: *Bothriochloa spp.* are invasive warm-season grasses invading native grasslands of the southern and central Great Plains, altering ecological services these grasslands supply. Our study investigated the potential allelopathic effects of the invasive grass species *B. ischaemum* on native grass germination, growth, and survival. Analyses for allelopathic compounds were also conducted.

Methods: Leachate or litter from *Andropogon gerardii* (native) or *B. ischaemum* were applied to two native grass species. Leachate and litter were also added to *B. ischaemum* and a water control was included. Germination, above- and belowground biomass, and survival were determined. Biochemical analyses were conducted on soils and leachates.

Results: Application of *B. ischaemum* leachate or litter significantly reduced the germination, growth, and survival of both *A. gerardii* and *S. scoparium* but had no conspecific effects, while *A. gerardii* treatments had no effect on any species. Biochemical analysis indicated *A. gerardii* leachate was greater in total phenolic content.

Conclusions: Bothriochloa spp. may gain a competitive advantage through the use of allelopathic biochemicals. However, it is unclear if these allelopathic effects directly hinder competitors or indirectly hinder them through alterations to soil microbial communities. Greater phenolic content in native leachates may be a mechanism for maintenance of plant biodiversity in native systems.

INTRODUCTION

Ecological systems have evolved under a constant state of stress. Stressors such as predation and/or competition ultimately shape the biological community of an ecosystem (Inouye et al. 1980; Olff and Ritchie 1998; Huitu et al. 2004). How these systems react to and balance these stressors ultimately determines the stability of the system and how likely they are to be restored if these stressors are altered or become overbearing. Global change has caused a shift in these ecological stressors through: 1) land transformations and loss; 2) alterations in global biogeochemistry; 3) climate change; and 4) alterations in biodiversity (Vitoussek et al. 1997). Plant communities are no exception to these global changes with ecological invasion by exotic plants species being at the forefront of research due to its ecological impacts. Through the cost of control or restoration of invaded sites or as direct loss or reduction of product (sustainable livestock and or grain production) biological invasions also generate tremendous economical impacts (Vitousek et al. 1997; Pimentel et al. 2000). Pimentel et al. (2005) has estimated the cost of control and lost or damaged goods due to invasive plants at approximately 35 billion dollars (for 25,000 exotic plant species) in the US.

Exotic species invasion has increased at unprecedented rates due to human activities that have amplified the number of introductions and the rate of spread for many of these species (Chapin et al. 2000). These invasions bring about changes in native species biodiversity (Heywood 1989), disturbance regimes (D'Antonio and Vitousek 1992) and ecological structure and functioning (Chapin et al. 2000). Due to these changes biological invasion by exotic plant

species is one of the major causes of native rangeland loss (Watkinson and Ormerod 2001) and global change (Vitousek et al. 1997). In the US loss of native prairies due to exotic plant invasions and increased land conversions rates, has left the grasslands of the Great Plains as one of the most endangered ecosystems in North America (Samson et al. 2004).

To help reduce soil erosion and to increase hay and forage production on marginal or deteriorated rangelands, a suite of warm-season perennial grasses were introduced into the southern and central Great Plains beginning in 1917 (McCoy et al. 1992, Harmoney et al. 2007). Old World Bluestems (*Bothriochloa spp.* and others) as this group collectively known as were introduced from areas of Europe, Asia, Russia, and Australia (McCoy et al. 1992; Harmoney et al. 2007). *Bothriochloa spp.* were selected for this introduction as they are capable of producing four times as much forage as native species in well managed rangelands (McCoy et al.1992). *Bothriochloa spp.* have been planted onto millions of hectares of marginal farmland, roadside and railroad right-of-ways, and Conservation Reserve Program (CRP) lands in the central and southern Great Plains (White and Dewald 1996; Harmoney and Hickman 2004). The benefits provided to ranchers by *Bothriochloa spp.* are short lived as they need to be harvested at appropriate times because forage quality and palatability declines rapidly as the plant matures (Dabo et al. 1988). There are also management concerns as *Bothriochloa spp.* have escaped their original planting sights and have started to invade native and native planted rangelands.

The link between soil and plants is circular in nature, as a change in one can alter the other which may then feed back to the first. This feedback process can be negative if the presence of a plant alters the soil or local environment in a way that slows the rate of population growth for that species over time (Bever et al. 1997; Casper et al. 2008). Negative feedbacks can lead to increased or stabilized diversity over time (Bever 2002; 2003) and are prominent in natural (native) systems (Packer and Clay 2000; Klironomos 2002; Bever 2003). Alternatively, these feedbacks can be positive if the relative growth rate of a plant community increases overtime with

the associated change in soil or local environment (Bever et al. 1997), and can decrease species diversity (Klironomos 2002; Reynolds et al. 2003) resulting in the spread and development of mono-specific stands.

How exotic species invade new habitat and create positive feedback loops has been previously studied and numerous hypotheses have been developed (see Mitchell et al. 2006). These hypotheses can be combined into four categories as they relate to explanation of exotic species: 1) the invader is released from an enemy; 2) the natives or competitors are hindered in some manner; 3) mutualisms are developed that aid the exotic; or 4) abiotic factors are altered in a manner that aids the exotic (Mitchell et al. 2006). These categories are not independent of each other as an alteration in one can lead to alterations in another.

Bothriochloa spp. are invading our native prairies and are doing so by outcompeting our native grass species, ultimately resulting in the production of positive feedback loops for themselves or negative feedback loops for native grasses (Schmidt et al. 2008; Wilson et al. 2012). It has been hypothesized that these feedback loops may be due to the high biomass production of these species, which increases the volatilization of N when burned and decreases the amount of N returned to the soil, creating an environment too low in nitrogen for even native grass species to survive (altering abiotic factors) (Reed et al. 2005). Positive feedback loops of *Bothriochloa spp.* may also alter soil communities in a way that increases mutualistic benefits for *Bothriochloa spp.* at the expense of benefits for native species (i.e. altering mutualisms) (Wilson et al. 2012). Wilson et al. (2012) hypothesized that a potential mechanism for *Bothriochloa spp.* success may be the production of allelopathic (or toxic) biochemicals by this invasive grass, thereby reducing competition. These allelopathic biochemicals could be directly toxic to the native grass species (Callaway and Ridenour 2004; Dorning and Cipollini 2006), or have indirect affects by inhibiting beneficial relationships with soil microbes such as arbuscular mycorrhizal

fungi (AMF), or with other plant species with similar evolutionary histories (Callaway and Aschehoug 2000; Stinson et al. 2006; Koch et al. 2011).

The use of "novel weapons" as described by Callaway and Ridenour (2004) may be a driving force in exotic plant invasion. Plants have been shown to produce 100,000 different biochemical products, many of which tend to be species-specific (Bais et al. 2002; 2003). These biochemicals evolved over long periods of time and may serve many purposes with similar biochemicals, performing different tasks based on geographic location and local habitat characteristics (Callaway and Ridenour 2004). These chemicals can be found in many plant tissues and can be released into the soil though tissue decomposition or directly through tissue exudates (Inderjit and Duke 2003). As exotic plants continue to spread into new habitats, biochemicals originally evolving for one purpose may act to perform a second purpose as native species are exposed to biochemicals they have never encountered before (Callaway and Ridenour 2004). If this secondary purpose has allelopathic properties, it may reduce or hinder the native species, ultimately giving the exotic plant species a competitive advantage.

Warm-season C_4 grasses such as *Bothriochloa spp.* have invaded the central and southern Great Plains, and are aggressively expanding their range into native grasslands (Reed et al. 2005; Baer et al. 2009). With current trends, policy, and practices, *Bothriochloa spp.* invasion will continue far into the future, bringing with it greater losses of native grasslands, displacement of native flora and fauna, leading to more complex problems and costly restorations. With the increased invasion by these exotic grasses there is a need to evaluate the underlying processes or mechanisms giving *Bothriochloa spp*. their advantage. To date, few mechanisms have been proposed as plausible causes for *Bothriochloa spp.* invasion and no empirical data have been reported evaluating allelopathic capabilities of these problematic grasses.

We conducted a greenhouse experiment to assess the potential allelopathic effects of *Bothriochloa ischaemum* (L.) Keng (yellow bluestem) on two dominant native grass species of the Great Plains. We hypothesized that *B. ischaemum* leachate or leaf litter will reduce the seed germination, above- and belowground biomass production, and seedling survival of native grasses, but these compounds will not have conspecific effects. We also hypothesized that leachate or litter from the native species *Andropogon gerardii* Vitman (big bluestem) would have neither conspecific nor heterospecific effects. If these hypotheses are supported, "chemical warfare" may be evident in nature and *Bothriochloa spp.* are achieving a competitive advantage over the native grasses of the Great Plains, at least partially, through the use of allelopathic biochemicals.

MATERIALS AND METHODS

General setup

Collection site: Seed, soil, and *B. ischaemum* and *A. gerardii* biomass were collected from the Oklahoma State University Range Research Station (OSU-SRR) in Payne county Oklahoma, which is located approxamatey 12 km west of Stillwater, OK, USA. Our collection site is located in the western part of the Cross Timbers ecosystem which occurs from southeastern Kansas to north central Texas. The Cross Timbers ecosystem is a mosaic of upland deciduous forest, savanna, and tallgrass prairie that forms a transition zone between the eastern deciduous forest and the grasslands of the southern Great Plains (van Els et al. 2010). Temperatures for the region vary greatly between seasons with summer temperatures averaging 34.0 °C and winter temperatures averaging -1.0 °C. Mean annual precipitation is 93.2 cm with a peak in rainfall normally occurring in May. Soils of this area very greatly with coarse-textured soils under the woodlands and fine-textured soils under the grasslands. The soils of this area tend to be loam dominated with pockets of fine sandy loam and silty loam (USDA NRCS 2007).

Seed collection: Seeds of two native prairie grasses: *A. gerardii* and *Schizachyrium scoparium* (Michx.) Nash (little bluestem) and the invasive grass *B. ischaemum* were collected locally from three representative sites at the OSU-SRR. *Andropogn gerardii* and *S. scoparium* were selected as representative of the dominant native warm-season grasses of the Great Plains. *Bothriochloa ischaemum* was selected, as this species is the dominant non-native invasive grass present at the OSU-SRR. Seeds were homogenized between collection sites to minimize seed collection site effects.

Leachate preparation: To produce the leachate used in the germination, growth, survival, and biochemical identification experiments, we collected actively growing (before seed emergence) whole plant biomass (roots and shoots) of both *A. gerardii* (native) and *B. ischaemum* (invasive) and manually removed soil from the roots system (without washing) (Roberts and Anderson 2001; Dorning and Cipollini 2006; Harnden et al. 2011). Plant material was collected from the same three sites at OSU-SSR as experimental seeds were collected. For each species, biomass was homogenized between collection sites before producing leachate for the germination, growth, and survival experiments. For biochemical identification, biomass collected from the three representative sites was kept separated by species and collection site to allow for statistical analysis. Leachate was produced by soaking freshly collected biomass in double-deionized water (dH$_2$O) (5 ml/g plant material) for 3 days at room temperature, with the assumption that the soaking process would capture root and shoot exudates (Roberts and Anderson 2001; Dorning and Cipollini 2006; Harnden et al. 2011). After three days, biomass was removed and the leachate was passed through cheesecloth to remove large debris (Harnden et al. 2011). To remove smaller debris and soil particles leachate was vacuum filtered through #42 Whatman® filter paper and stored at 4 °C until use (Dorning and Cipollini 2006).

Soil collection and seedling establishment: For the survival and growth experiment, we collected soil from (0-20 cm) native prairie from the OSU-SSR. Soil was transported to the

Oklahoma State University soil processing room, sieved to remove large debris and rocks and homogenized before plastic pots (14 cm tall x 11 cm wide) were filled with 1 kg (dry wt) of soil. At initiation of the study, we assed soil pH, plant-available P (Mehlich test 3), and NO_3. Soil samples were analyzed by the Oklahoma State University Soil, Water and Forage Analytical Laboratory, Stillwater, OK. At initiation of the survival and growth experiment, soils had a pH 7.8, with 1.25 ppm plant-available P, and 12.75 ppm NO_3.

Seeds of all three species (*A. gerardii*, *S. scoparium*, and *B. ischaemum*) were grown in separate seedling flats containing sterile vermiculite for 14 days. After 14 days, (second leaf stage) seedlings were transplanted into the plastic pots containing native prairie soil mentioned above. Transplanted seedlings were given 1 week to establish before treatments were applied. Pots were randomly placed throughout the greenhouse to avoid position effects (Kardol et al. 2007). All pots were placed on shallow saucers to help collect leachate or water that ran through the soil before it could be absorbed allowing the plant/soil to absorb it as needed (Koch et al. 2011). Any seedling that died during this establishment period was replaced with a new seedling that was established at the same time as all other seedlings. The use of "back-up" pots allowed us to eliminate variability caused by differences in planting times if original pots were replanted during the establishment period.

Germination experiment

This experiment tested the effects of leachate collected from native (*A. gerardii)* or invasive grasses (*B. ischaemum*) on seed germination of *A. gerardii*, *S. scoparium*, or *B. ischaemum*. Seeds were surface sterilized by soaking in 7% sodium hypochlorite solution for 10 minutes and then rinsing thoroughly with distilled water (Ruiz et al. 2003). For each species, 50 seeds were placed into each of 24 (3 treatments x 8 reps), sterile plastic petri dishes (100 mm diameter x15 mm height) containing a double layer of #1 Whatman® filter paper (Keeley and

Fotheringham 1998). Five ml of dH$_2$O (control) or leachate from *A. gerardii* or *B. ischaemum* were added to each of the 8 replicate dishes. All dishes were maintained in a controlled environmental chamber (Conviron- PGW 36) under a photoperiod of 16 hours in light at 30 °C and 8 hours in dark at 20 °C. Location of the replicate petri dishes was randomized within the environmental chamber to avoid biases associated with location within the chamber (Kardol et al. 2007).

Germination measurements were initiated one week after experimental set-up. At this time, germination was recorded every other day for 14 days, at which time no new germinates emerged. A seed was considered to have germinated when the protruding radical achieved the length of 2 mm beyond the seed coat (Beligni and Lamattina 2000). Each germinated seedling was removed after it was counted (Harnden et al. 2011). An additional 2 ml of water or extract was added every six days to each dish as the filter paper began to dry.

Growth and survival experiment

This experiment tested the possible allelopathic effects of leachate or leaf litter collected from native (*A. gerardii)* or invasive grasses (*B. ischaemum*) on growth and survivorship of *A. gerardii*, *S. scoparium*, or *B. ischaemum*. After completion of the establishment period seedlings of all three species were randomly assigned to one of 5 treatments: 1) control (no litter or leachate additions); 2) *A. gerardii* leachate addition; 3) *B. ischaemum* leachate addition; 4) *A. gerardii* leaf litter addition; 5) *B. ischaemum* leaf litter addition. For each leachate treatment, 60 ml of leachate was added to each of the 8 replicate pots for each of the three species (*A. gerardii, S. scoparium, B. ischaemum*) at experiment initiation and every 2 weeks until experiment completion (Koch et al. 2011). Fresh aboveground biomass was collected from the three representative collections sites at OSU-SRR, clipped into small pieces (1-3 cm), homogenized within species, and applied around seedlings to a depth of 20 cm (equivalent to the litter depth of the native prairie collection

site) for each of the 8 replicate pots for each of the three grass species. All treatments were watered with dH$_2$O every other day or as needed during warmer temperatures (Dorning and Cipollini 2006).

After 12 weeks, whole plant biomass was harvested and separated into above- and belowground portions. Roots were washed free of soil, biomass was dried (60 °C for 72 hrs), and weighed. Plant survival was assessed every day for the first 2 weeks, and twice weekly thereafter.

Biochemical identification

To determine what biochemicals may be attributing to the allelopathic effects exhibited by *B. ischaemum* and that may ultimately be leading to the rapid spread of this species, we preformed tests on the soil collected from beneath all species x treatment combinations at the end of the 12 week experiment, and on the *A. gerardii* and *B. ischaemum* leachates. For each of the species by site treatment combinations, we sub-sampled soil from three replicate pots for pH and soil salinity via electrical conductivity (EC) (uS/cm) analysis. Soil pH was measured with a pH meter using a solid/double-deionized (dH$_2$O) water ratio of 1:3 (5 g soil added to 15 ml dH$_2$O) modified from (Hardie and Doyle 2012). Electrical conductivity was measured in the same solution using a separate EC meter (Accument AB30; Fisher Scientific, Pittsburgh, PA).

To further assess potential biochemicals attributing to the allelopathic affects of *B. ischaemum*, we assessed properties of three replicate samples of both the *A. gerardii* and *B. ischaemum* leachate. We measured pH of leachate from each species using a pH meter and analyzed for maximum absorbance using a spectrophotometer (Beckman Couter DU730, Beckman Coulter, Irving, TX). *B. ischaemum* leachate was characterized by a distinctive yellow brown color. To determine if this color (and possible *B. ischaemum* allelopathic properties) was attributed to organic or aqueous soluble compounds, equal parts of leachate and chloroform were added to a clean dry test tube, shaken vigorously, and monitored for 5 min (Jones and Fleming

2010). As phenols have been shown to inhibit numerous plant processes (Einhellig and Rasmussen 1979), they are considered to possess allelopathic potential (Whittaker and Feeny 1971; Rice 1974). Therefore, we tested leachate from *A. gerardii* and *B. ischaemum* for total phenolic content. Total phenolic content were measured by the Folin-Ciocalteau (FC) method (Singleton & Rossi 1965), based on the chemical reduction of tungsten and molybdenum oxides. We combined 0.2 ml of the leachate solution with 15.8 ml of *d*H_2O water, 1 ml of Folin-Ciocalteau (FC) reagent and 3 ml of sodium carbonate in a 40 ml test tub. A phenolic concentration standard curve was generated with tannic acid (concentrations between 0 to 50 mg/l), to better fit the standard curve leachates were diluted to 8x their original concentrations. Both standards and samples were analyzed in a spectrophotometer (Spectronic 21D, Milton Roy) set to 765 nm. Therefore total phenols (mg/l) are reported as tannic acid equivalents. Finally, leachates were analyzed for elemental concentrations (mg/l) of the following Na, Ca, Mg, K, S, B, P, Fe, Zn, Cu, Mn, Al, Mo, As, Cd, Co, Cr, and Pb by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Statistical analysis

Germination and seedling survival data are reported as percent of the control (seeds and seedlings amended with distilled water) to show the relative effects of the biochemical compounds on native and invasive species. Growth data are reported as above- and belowground biomass production. Two-sample t-tests were used to assess differences between *A. gerardii* and *B. ischaemum* leachate on seed germination and *A. gerardii* and *B. ischaemum* leachate or litter on seedling survival at alpha = 0.05 (Freund and Wilson 2003). We used two seperate one-way analysis of variances (ANOVA) to assess potential differences between the control (*d*H_2O), *A. gerardii* leachate, and *B. ischaemum* leachate on our three study species, post-hoc differences were assessed using a Tukeys test at an alpha of 0.05. Two-sample t-tests were used to determine effects of *A. gerardii* litter and *B. ischaemum* litter (alpha = 0.05) on *A. gerardii, S. scoparium, B.*

ischaemum seedlings. The dH_2O control was not applicable to this study, as dH_2O was applied to all litter treatments (Freund and Wilson 2003). Soil pH and EC were assesed with two seperate two-way analysis of variances (alpha = 0.05) for plant species (*A. gerardii*, *S. scoparium*, *B. ischaemum*) x treatment (control [dH_2O], *A. gerardii* leachate, *B. ischaemum* leachate, *A. gerardii* leaf litter, *B. ischaemum* leaf litter). The interaction of species x treatment was not significat, therefore soil pH and EC were reanalysed with a single factor analysis of variances for each treatment. Between treatment differences were determined post-hoc with a Tukey's test at an alpha = 0.05 (Freund and Wilson 2003). Initial pH values were measured at the initiation and at the conclusion of the study and these values were compared to assess for shifts in pH during the experiment. Leachate chemical properties were subjected to two sample t-tests (alpha = 0.05) to determine if differeces existed between *A. gerardii* and *B. ischaemum* leachates. All analyses were completed using R (R Core Team 2012) and MASS (Venables and Ripley 2002) and pscl (Jackman 2012) packages for basic calculations.

RESULTS

Germination experiment

Application of *A. gerardii* leachate had no effect on the germination of any of the three grass species (Fig. 1). However, germination of both native grass species (*A. gerardii* and *S, scoparium)* was substantially and significantly reduced following application of *B. ischaemum*. However, we did not observe a reduction in percent germination of *B. ischaemum* following the application either leachate on this invasive grass (Fig. 1).

Growth and survival experiment

Growth: Above- and belowground biomass production were significantly reduced for all three species by the application of *B. ischaemum* leachate, as compared to the control (Fig. 2a, b). Applications of *A. gerardii* leachate had no significant effects on *A. gerardii* or *S. scoparium, as*

compared to each corresponding control. However, applications of *A. gerardii* leachate significantly reduced above- and belowground biomass production of *B. ischaemum* (Fig. 2a, b). *B. ischaemum* leachate had a substantially greater negative effect on the native grasses, *A. gerardii* and *S. scoparium*, than applications of *A. gerardii* leachate (Fig. 2a, b). Above- and belowground biomass production of *B. ischaemum* was significantly reduced, compared to the corresponding *d*H_2O control, following applications of *A. gerardii* or *B. ischaemum* leachate.

Application of either *A. gerardii* or *B. ischaemum* litter reduced the above- and belowground biomass production of all three species (Fig. 3a, b). However, *B. ischaemum* litter exhibited a larger negative effect overall (Fig. 3a, b). For all three species, above- and belowground biomass production was significantly different between the *A. gerardii* and *B. ischaemum* leaf litter applications, with reduction of biomass being significantly greater following *B. ischaemum* leaf litter applications as compared to *A. gerardii* litter applications (Fig 3a, b).

Survival: Plant survival was not affected by applications of *A. gerardii* leachate or litter for any species (Fig. 4a, b). However, application of *B. ischaemum* leachate or litter reduced plant survival of both *A. gerardii* and *S. scoparium* (Fig. 4a, b). However, reductions in *B. ischaemum* survival following applications of *B. ischaemum* litter were not observed. *B. ischaemum* leachate had a larger negative effect on the native grasses, as compared to *B. ischaemum* litter (Fig. 4a, b). Plant survivorship of the native grasses, *A. gerardii* and *S. scoparium*, were profoundly and significantly reduced following applications of either *B. ischaemum* leachate or leaf litter, with little to no survivorship of these native species (Fig 4a, b). However, plant survival of *B. ischaemum* was not affected by either *A. gerardii* or *B. ischaemum* leachate or leaf litter (Fig 4a, b).

Biochemical identification

Soil measurements: No species x treatment interactions were significant for either pH or EC, so species were combined before analysis. Values for pH ranged from 7.600–8.270 across all samples (both pre- and post- experiment assessments) with means ranging from 7.783–8.245 (Table 1). pH was not significantly different (F=2.212; p=0.121) between the control or *A. gerardii* and *B. ischaemum* leachate or leaf litter. Soil salinity (EC) readings ranged from 145.43–469.83 (Table 2) with means ranging from 150.26–289.92. There were no significant differences (F=1.840; p=0.198) in soil salinity (EC) between *A. gerardii* and *B. ischaemum* leachate or leaf litter or each corresponding control.

Leachate measurements: Maximum absorbency or pH of leachate solutions revealed no significant differences between *A. gerardii* and *B. ischaemum* leachate (Table 3). Aqueous/organic solubility did not differ between *A. gerardii* and *B. ischaemum* leachate, with the colored portion of the solution remaining aqueous soluble (Table 3). *A. gerardii* leachate was significantly greater in phenolic content, compared to *B. ischaemum* leachate (Table 3). Comparison of the elemental composition of the leachates (Na, Ca, Mg, K, S, B, P, Fe, Zn, Cu, Mn, Al, Mo, As, Cd, Co, Cr, and Pb) indicated than there were no significant differences for any of the elements in *A. gerardii* or *B. ischaemum* leachates (Table 3). Several elements common to soils (Mo, As, Cd, Co, Cr, and Pb) contained concentrations below our detection limit.

DISCUSSION

Numerous hypotheses have been proposed as to what may give invasive species their competitive advantage over native species. Mitchell et al. (2006) summarized these hypotheses and grouped them into four categories (enemy, mutualisms, competition, abiotic) based on ecological interactions, how invasive species influence the local environment, or how environment influences invasive and native species. These hypotheses are not independent of

each other; it is possible that many of them work synergistically to aid invasive species in expansion of their range (Mitchell et al. 2006). Our study evaluated potential utilization of "novel weapons" (Callaway and Aschehoug 2000; Callaway and Ridenour 2004) (i.e. allelopathic biochemicals) by *Bothriochloa spp.* to gain a competitive advantage over native warm-season greases. Our results support the "novel weapons" hypotheses as germination, biomass (above- and belowground) production, and survival of native grass species were significantly reduced by the application of *B. ischaemum* leachate or litter, but few negative conspecific effects were experienced by *B. ischaemum* grown under the same treatments. To our knowledge, this study is the first to provide empirical data illustrating allelopathic potential of leachate and litter of an invasive grass on native grass species. Dirvi and Hussain (1979) reported allelopathic effects of *Dichanthium annulatum* (Forssk.) Stapf, a species that is closely related to *Bothriochloa spp.*, on species of cultivated crops in Pakistan, an area where *D. annulatum* is native, but no data are available assessing allelopathic potential of invasive grasses expanding into native grasslands.

Several previous studies have shown invasive species may release exudates that directly kill native species or prevent native species from germinating or establishing (Grove et al. 2012; Hu and Zhang 2013). Native plant species have been hypothesized to associate with soil microbes that have evolved with native plant-produced compounds, resulting in the selection of microbial species capable of degrading these native produced compounds (Inderjit and van der Putten 2010). If native soil microbes are exposed to novel biochemicals they have not evolved with, such as those produced by non-native plant species, their inability to degrade these chemicals could result in accumulation to toxic levels. Accumulation of such compounds could have led to the reduction in germination, growth, and survival of the native species in our experiment. Our results support these hypotheses, as not only were the native species (*A. gerardii* and *S. scoparium*) germination, growth, and survival reduced by addition of foreign compounds (leachate or degradation of leaf litter) from *B. ischaemum*, but addition of 'foreign compounds'

from *A. gerardii* (leachate or degradation of leaf litter) to *B. ischaemum* soils reduced biomass (above- and belowground) production of *B. ischaemum*. The reduction of all species biomass production by addition of 'foreign compounds' indicates that coevolution with the soil microbial community may play a vital role in invasibility of some invasive species.

Generally, native warm-season grasses are dependent on arbuscular mycorrhizal (AM) fungi to complete their life cycles (Wilson and Hartnett 1998). These native grasses form vast networks of AM fungi that link numerous individuals together allowing them to transport nutrients between linked individuals (Wilson et al. 2006). Invasive species have been shown to release chemical exudates that disrupt symbiotic relationships with soil microbes, directly inhibiting the ability of AM fungi to colonize these native grasses, or indirectly reducing AM fungal colonization by suppressing the growth of the native grasses, thereby reducing carbon allocations to the symbiont (Roberts and Anderson 2001; Stinson et al. 2006; Abhilasha et al. 2008; Callaway et al. 2008; Inderjit & van der Putten 2010; Grove et al. 2012). Disruption of these coevolved networks could lead to a reduction in benefits supplied to the native grass species, ultimately leading to increased invasibility potential by *Bothriochloa spp.* Chemical exudates released by invasive species may also have potential to influence the composition of the soil microbial community (Cipollini et al. 2012; Zhou et al. 2013). Disruption of species-specific associations between native grasses and their AM fungal partners could again result in a reduction of benefits to the native grass species, increasing the potential for *Bothriochloa spp.* to invade native grasslands. Wilson et al. (2012) observed greater levels of root colonization and extra-radical hyphae in the soil surrounding invasive grass roots, as compared to native plants and soil, and they hypothesized that this increase in AM fungi may be due to a shift from specialized AM species associating with native grass species to a suite of generalist AM species that associate with *Bothriochloa spp.* The reduction in germination, growth, and survival after application of either *B. ischaemum* leachate or leaf litter may be in response to a shift in AM fungi species.

Neither Wilson et al. (2012) nor our study were able to determine if the non-native invasive plant species altered the composition of the AM fungal communities, as molecular analysis of roots and the surrounding soil was not conducted in either study. These analyses would provide valuable insight in determining if the AM fungal communities associating with the invasive grasses differed from those associating with native species. While molecular analysis was beyond the scope of this study, M. Greer (personal communication, Oklahoma State University) is conducting these assessments through analyses of field-collected roots and soils for several of the invasive and native warm-season grasses of the central and southern Great Plains. Given the results of this proposed research future research should assess direct affects of the allelopathic biochemical on the AM fugal communities.

The use of allelopathic chemicals by invasive species to expand their range in new environments has been previously documented (e.g. Prati and Bossdorf 2004; Dorning and Cipollini 2006; Harden et al. 2011), but in many of these studies the invader experienced little to no effect following application of the leachates. In our study, above- and belowground biomass of all species, including *B. ischaemum*, was reduced by addition of *B. ischaemum* leachate or litter. However, biomass production was the only plant trait in which application of *B. ischaemum* treatments resulted in reduced performance of *B. ischaemum* itself. Both percent seed germination and seedling survival rate for *B. ischaemum* were unaffected by application of *B. ischaemum* leachate or litter. This reduction in biomass production indicates that the use of allelopathic compounds is not without direct cost to *B. ischaemum*. However, our results do indicate that competition may be reduced through the reduction of germination and survival of native grasses due to production of allelopathic compounds. This competitive advantage may result in long-term benefits with increased invasibility for *B. ischaemum.* This reduction in heterospecific competitors increases the "competitive ability" (Blossey and Nötzgold 1995; Bossdorff et al. 2005) of *B. ischaemum* and creates more opportunities for invasion ("Invasion Opportunity

Windows") (Johnstone 1986; Agrawal et al. 2005). Use of "novel weapons" (Callaway and Aschehoug 2000; Callaway and Ridenour 2004) by *B. ischaemum* to eliminate competitors illustrates that the invasion hypotheses summarized in Mitchell et al. (2006) are in fact not mutually exclusive, but are synergistic in nature by creating or altering feedbacks loops that further enhance the success of the invasive species at the expense of the native (Wootton 1994, Bever 2003; Mitchell et al. 2006).

Above- and belowground biomass production of each of the three grass species was reduced in response to litter addition, regardless of litter origin (*A. gerardii* or *B. ischaemum*), compared to biomass production of these species in the absence of litter, with no leachate amendments. These results were not unexpected, as reduced seedling growth due to shading from litter at early growth stages has been observed in both greenhouse (Evans 1961) and field studies (Jameson 1966; Hulbert 1969). Both leachate and litter applications of *B. ischaemum* profoundly reduced the production and survival of native grasses which indicates more than just a shading effect is driving reduction in biomass production of the native and invasive grasses.

Use of allelopathic chemicals by *Bothriochloa spp.* has possible implications for the restoration of invaded grounds. A recent study by Greer (2013), indicated plant growth suppression following invasion by some non-natives is, at least partially, through the alteration in soil microbial communities and that these communities could be restored through soil sterilization and re-inoculation with native soil amendments. However, if the allelopathic chemicals produced by *Bothriochloa spp.* cannot be degraded by the native microbial community and remain in the soil, inoculating with native microbial communities would not be expected to result in a positive growth response by native grasses. Therefore, it may be of utmost importance to determine the identity of allelopathic compounds present, how to counteract negative effects, and length of residency time following invasive species removal.

Determination of potential allelopathic biochemicals is difficult, as chemical isolation of compounds from soils or dead plant materials may differ substantially in form and function from compounds extracted from living tissues (Hagen et al. 2013). It is also difficult to identify individual compounds as the driving allelopathic biochemical, as several compounds may work in combination (Hierro and Callaway 2003). Furthermore, if potential allelopathic chemicals are identified, their function in the plant soil system may not be fully understood (Uren 2007). Our study is the first to assess potential allelopathic compounds in invasive warm-season grasses. Soil collected from stands of *A. gerardii* or *B. ischaemum* were not significantly different in pH or soil salinity (EC). However, the lack of significance could be a reflection of the difficulties in detecting biologically significant concentrations of allelopathic compounds in the soil (Hagan et al. 2013). Further biochemical analysis indicated little difference between *A. gerardii* and *B. ischaemum* leachate. Maximum absorbency of both leachates exhibited readings at ~280 nm, indicating the presence of proteins (Coulter et al. 1936). While aqueous and organic solubility were similar for *A. gerardii* and *B. ischaemum* leachate, it is possible that the allelopathic biochemicals are not pigmented, and may be organic soluble.

Our biochemical analyses indicated total phenolic content of *A. gerardii* leachate was greater than that of *B. ischaemum* leachate. Phenols have been shown to possess growth suppressant properties (Einhellig and Rassmussen 1979; Muscolo et al. 2013), direct toxicity to some organisms (Chon and Kim 2002), and ability to alter soil nutrient availability (Blum et al. 1993). Therefore, that leachate produced from native grasses would possess greater total phenolic content than that of invasive grasses seems counterintuitive. However, it has been hypothesized that negative feedbacks are prominent in native ecosystems (Packer and Clay 2000; Klironomos 2002; Bever 2003). These negative feedbacks alter soil microbial communities, local environment, or plant communities in a manner that slows population growth rates of native species (Bever et al. 1997; Casper et al. 2008) and can lead to increased or stabilized plant

diversity over time (Bever 2002; 2003). Therefore, if native ecosystems operate under negative feedbacks, the greater amount of total phenolic content in the native *A. gerardii* leachate, compared to that produced from the invasive *B. ischaemum*, may be a driving factor resulting in native ecosystem stability. However, with over 100 secondary compounds currently identified as plant tissue exudates (Uren 2007), it is likely that more than one compound is driving these negative feedbacks.

Our results support our hypotheses that invasive grasses have potential to create positive feedbacks through the use of allelopathic compounds that reduce native seed germination and seedling survival with few conspecific effects. The reduction of native competitors may open new windows of opportunity allowing *B. ischaemum* to continue invading the grasslands of the southern and central Great Plains. While *Bothriochloa spp*. have shown potential for use of allelopathic chemicals, this species and other invasive species may suppress native plant growth through additional mechanisms, such as alterations in soil biotic communities. It is also possible that these two mechanisms are not mutually exclusive and work synergistically to aid invasion success of native grasslands. It is important to understand the mechanisms driving invasion by exotic plant species to best direct restoration efforts after the removal of exotic species.

REFERENCES

Abhilasha D, Quintana N, Vivanco J, Joshi J (2008) Do allelopathic compounds in invasive *Solidago canadensis* s.I. restrain the native European flora? J Ecol 96:993-1001

Agrawal AA, Kotanen PM, Mitchell CE, Power AG, Godsoe W, Klironomos J (2005) Enemy Release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology 86:2979-2989

Baer SG, Engle DM, Knops JMH, Langeland KA, Maxwell BD, Menalled FD, Symstand AJ (2009) Vulnerability of rehabilitated agricultural production systems to invasion by nontarget plant species. Environ Manage 43:189-196

Bais HP, Walker TS, Stermitz FR, Hufbauer RA, Vivanco (2002) Enantiomeric-dependent phytotoxic and antimicrobial activity of (+/–)-catechin. A rhizosecreted racemic mixture from spotted knapweed. Plant Physiol 128: 1173-79

Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM (2003) Allelopathy and exotic plants: From genes to invasion. Science 301:1377-1380

Beligni MV, Lamattina L (2000) Nitric oxide stimulates seed germination and de0etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta 210:215-221

Bever JD (2002) Negative feedback within a mutualism: Host-specific growth of mycorrhizal fungi reduces plant benefit. P R Soc London 269:2595-2601

Bever JD (2003) Soil community feedback and the coexistence of competitors: Conceptual frameworks and empirical test. New Phytol 157:465-473

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: The utility of the feedback approach. Ecology 85:561-573

Blossey B, Nötzgold R (1995) Evolution of increased competitive ability in invasive non-indigenous plants: A hypothesis. J Ecol 83:887-889

Blum U, Gerig TM, Worsham AD, King LD (1993) Modification of allelopathic effects of p-coumaric acid on morning-glory seedling biomass by glucose, methionine, and nitrate. J Chem Ecol 19:2791-2811

Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic variation between native and introduced plant population. Oecologia 144:1-11

Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science 290:521-523

Callaway RM, Cipollini D, Barto K, Thelen GC, Hallett SG, Prati D, Stinson K, Klironomos J (2008) Novel weapons: Invasive plant suppresses fungal mutualisms in America but not in its native Europe. Ecology 89:1043-1055

Callaway RM, Ridenour WM (2004) Novel weapons: Invasive success and the evolution of increased competitive ability. Front Ecol Environ 2:436-433

Casper BB, Bentivenga SP, Ji B, Doherty JH, Edenborn HM, Gustafson DJ (2008) Plant-soil feedback: Testing the generality with the same grasses in serpentine and prairie soils. Ecology 89:2154-2164

Chapin FS III, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU (2000) Consequences of changing biodiversity. Nature 405:234-242

Chon SU, Kim JD (2002) Biological activity and quantification of suspected allelochemicals from alfalfa parts. J Agron Crop Sci 188:281-285

Cipollini D, Rigsby DM, Barto EK (2012) Microbes as targets and mediators of allelopathy in plants. J Chem Ecol 38:714-727

Coulter CB, Stone FM, Kabat EA (1936) The structure of ultraviolet absorption spectra of certain proteins and amino acids. J Gen Physiol 19:739-752

D'Antonio CM, Vitousek PM (1992) Biological invasion by exotic grasses, the grass-fire cycle, and global change. Annu Rev Ecol Syst 23:63-87

Dabo SM, Taliaferro CM, Coleman SW, Horn FP, Claypool PL (1988) Chemical composition of old world bluestem grasses as affected by cultivar and maturity. J Range Manage 41:40-48

Dirvi GA, Hussain F (1979) Allelopathic effects of *Dichanthium annulatum* (Forsk) Stapf on some cultivated plants. Pakistan J Sci Ind R 22:194-197

Dorning M, Cipollini D (2006) Leaf and root extracts of the invasive shrub, *Lonicera maackii*, inhibit seed and germination of three herbs with no autotoxic effects. Plant Ecol 184:287-296

Einhellig FA, Rassmussen JA (1979) Effects of three phenolic acids on chlorophyll content and growth of soybean and grain sorghum seedlings. J Chem Ecol 5:815-824

Evans RA (1961) Effects of different densities of downy brome (*Bromus tectorum*) on growth and survival of crested wheatgrass (*Agropyron desertorum*) in the greenhouse. Weeds 9:216-223

Freund RJ, Wilson WJ (2003) Statistical methods, 2nd ed. Academic Press, CA

Greer MJ (2013) Grassland invasions by non-native grass species: Ecological issues for multiple species at multiple trophic levels. Dissertation, Oklahoma State University

Grove S, Haubensak KA, Parker IM (2012) Direct and indirect effects of allelopathy in the soil legacy of an exotic plant invasion. Plant Ecol 213:1869-1882

Hagen DL, Jose S, Lin C-H (2013) Allelopathic exudates of cogongrass (*Imperata cylindrical*): Implications for performance of native pine savanna plant species in the southeastern U.S. Journal of Chem Ecol 39:312-322

Hardie M, Doyle R (2012) Measuring soil salinity. Methods Mol Biol 913:415-425

Harmoney KR, Hickman KR (2004) Comparing morphology of caucasian old world bluestem and native grass. Agon J 96:1540-1544

Harmoney KR, Stahlman PW, Hickman KR (2007) Suppression of caucasian old world bluestem with split application of herbicides. Weed Technol 21:573-577

Harnden J, MacDougall AS, Sikes BA (2011) Field-based effects of allelopathy in invaded tallgrass prairie. Botany 89:227-234

Heywood VH (1989) Patterns, extents and modes of invasions by terrestrial plants. In: Drake JA, Mooney HA, di Castri F, Grovers RH, Kruger FJ, Rejmanek M, Williamson M (eds) Biological invasions: a global perspective. Wiley, Chichester, pp 31-60

Hierro JL, Callaway RM (2003) Allelopathy and exotic plant invasion. Plant Soil 256:29-39

Hu G, Zhang ZH (2013) Allelopathic effects of *Chromolaena odorata* on native and non-native herbs. J Food Agric Environ 11:878-882

Hulbert LC (1969) Fire and litter effects in undisturbed bluestem prairie in Kansas. Ecology 5:874-877

Huitu O, Norrdahl K, Korpimäki (2004) Competition, predation and interspecific synchrony in cyclic small mammal communities. Ecography 27:197-206

Inderjit, Duke S (2003) Ecophysiological aspects of allelopathy. Planta 217:529-539

Inderjit, van der Putten WH (2010) Impacts of soil microbial communities on exotic plant invasion. Trends Ecol Evol 25:512-519

Inouye RS, Byers GS, Brown JH (1980) Effects of predation and competition on survivorship, fecundity, and community structure of desert annuals. Ecology 61:1344-1351

Jackman S (2012) pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory, Stanford University. Department of Political Science, Stanford University. Stanford, California. R package version 1.04.4

Jameson DA (1966) Pinyon-juniper litter reduces growth of blue grama. J Range Manage 4:214-217

Johnstone IM (1986) Plant invasion windows: A time-based classification of invasion potential. Biol Rev 61:369-394

Jones M, Fleming SA (2010) Organic Chemistry, 4th edn. Norton, New York

Kardol P, Cornips NJ, van Kempen MLL, Tanja Bakx-Schotman JM, Van der Putten WH (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77:147-162

Keeley JE, Fotheringham CJ (1998) Smoke-induced seed germination in California chaparral. Ecology 79:2320-2336

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Lett Nat 417:67-70

Koch AM, Antunes PM, Barto EK, Cipollini D, Mummey DL, Klironomos JN (2011) The effects od arbuscular mycorrhizal (AM) fungal and garlic mustard introductions on native AM fungal diversity. Biol Invasions 13:1627-1639

McCoy SD, Mosley JC, Engle DM (1992) Old world bluestem in western Oklahoma. Rangelands 14:41-44

Mitchell CE, Agrawal AA, Bever JD, Gilbert GS, Hufbauer RA, Klironomos JN, Maron JL, Morris WF, Parker IM, Power AG, Seabloom EW, Torchin ME, Vázquez (2006) Biotic interactions and plant invasions. Ecol Lett 9:726-740

Muscolo A, Sidari M, Texeira da Silva JA (2013) Biological effects of water-soluble soil phenol and soil humic extracts on plant systems. Acta Physiologiae Plantarum 35:309-320

Olff H, Ritchie ME (1998) Effects of herbivores on grassland plant diversity. Tree 13:261-265

Packer A, Clay K (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature 404:278-281

Pimentel D, Lach L, Zuniga R, Morrison D. (2000) Environmental and economic costs of nonindigenous species in the United States. Bioscience 50:53-65

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273-288

Prati D, Bossdorf O (2004) Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). Am J Bot 91:285-288

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reed HE, Seastedt TR, Blair JM (2005) Ecological consequences of C4 grass invasion of a C4 grassland: A dilemma for management. Ecol Appl 15:1560-1569

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grass roots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84:2281-2291

Rice, EL (1974) Allelopathy. Academic Press, New York

Roberts KJ, Anderson RC (2001) Effects of garlic mustard (*Alliaria petiolata* [Bieb. Cavara & Grande]) extracts on plants and arbuscular mycorrhizal (AM) fungi. Am Midl Nat 146:146-152

Ruiz ON, Hussein HS, Terry N, Daniell H (2003) Phytoremediation of organomercurial compounds vis chloroplast genetic engineering. Plant Physiol 132:1344-1352

Samson FB, Knopf FL, Ostlie WR (2004) Great Plains ecosystem: past, present and future. Wildlife Soc B 32:6-15

Schmidt CD, Hickman KR, Channell R, Harmoney K, Stark W (2008) Competitive abilities of native grasses and non-native (*Bothriochloa* spp.) grasses. Plant Ecol 197:69-80

Singleton VL, Rossi JA Jr (1965) Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am J Enol Viticult 16:144-158

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture (2007) Web Soil Survey. http://websoilsurvey.nrcs.usda.gov/. Accessed 13 September 2013

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms Plos Biol 4:727-731

Uren NC (2007) Types, amounts, and possible functions of compounds released into rhizosphere by soil-grown plants. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere: Biochemistry and organic substances at the soil-plant interface, 2nd edn. CRC Press, Boca Raton, FL, pp 1-21

van Els P, Will RE, Palmer MW, Hickman KR (2010) Changes in forest understory with *Juniperus* in OK, USA. Appl Veg Sci 13:356-368

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York

Vitousek PM, Mooney HA, Lubchenco J, Melillo M (1997) Human domination of earth's ecosystems. Science 277:494-499

Watkinson AR, Ormerod SJ (2001) Grasslands, grazing and biodiversity: editors' introduction. J Appl Ecol 38:233-237

White LM, Dewald CL (1996) Yield and Quality of WW-Iron Master and caucasian bluestem regrowth. J Range Manage 49:42-45

Whittaker RJ, Feeny PP (1971) Allelochemics: Chemical interactions between species. Science 171:757-770

Wilson GW, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in prairie grasses and forbs. Am J Bot 85:1732-1738

Wilson GWT, Hartnett DC, Rice CW (2006) Mycorrhizal-mediated phosphorus transfer between the tallgrass prairie plants *Sorghastrum nutans* and *Artemisia ludoviciana*. Funct Ecol 20:427-435

Wilson GWT, Hickman KR, Williamson MM (2012) Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. Mycorrhiza 22:327-336

Wootton JT (1994) The nature and consequences of indirect effects in ecological communities. Annu Rev Ecol Evol S 25:443-466

Zhou B, Kong C-H, Li Y-H, Wang P, Xu X-H (2013) Crabgrass (*Digitaria sanguinalis*) allelochemiclas that interfere with crop growth and soil microbial community. J Agr Food Chem 61:5310-5317

TABLES

Table 1. Mean, minimum, maximum, and standard errors of pH values determined for field-collected soil at initiation (initial) of study and from soil collected beneath plants of all soil treatments (control, *A. gerardii* and *B. ischaemum* leachate and leaf litter) at completion of study (12 weeks).

Treatment	Mean	Min.	Max.	SE
Initial	7.783	7.600	7.900	0.093
Control	7.962	7.909	7.991	0.027
A. gerardii Leachate	8.074	8.009	8.159	0.045
B. ischaemum Leachate	8.062	7.979	8.209	0.074
A. gerardii Leaf Litter	8.245	8.206	8.270	0.020
B. ischaemum Leaf Litter	7.840	7.671	7.988	0.092

Table 2. Mean, minimum, maximum, and standard errors values of soil salinity measured as electro-conductivity (uS/cm) determined from soil collected beneath plants of all soil treatments (control, *A. gerardii* and *B. ischaemum* leachate and leaf litter) at completion of study (12 weeks).

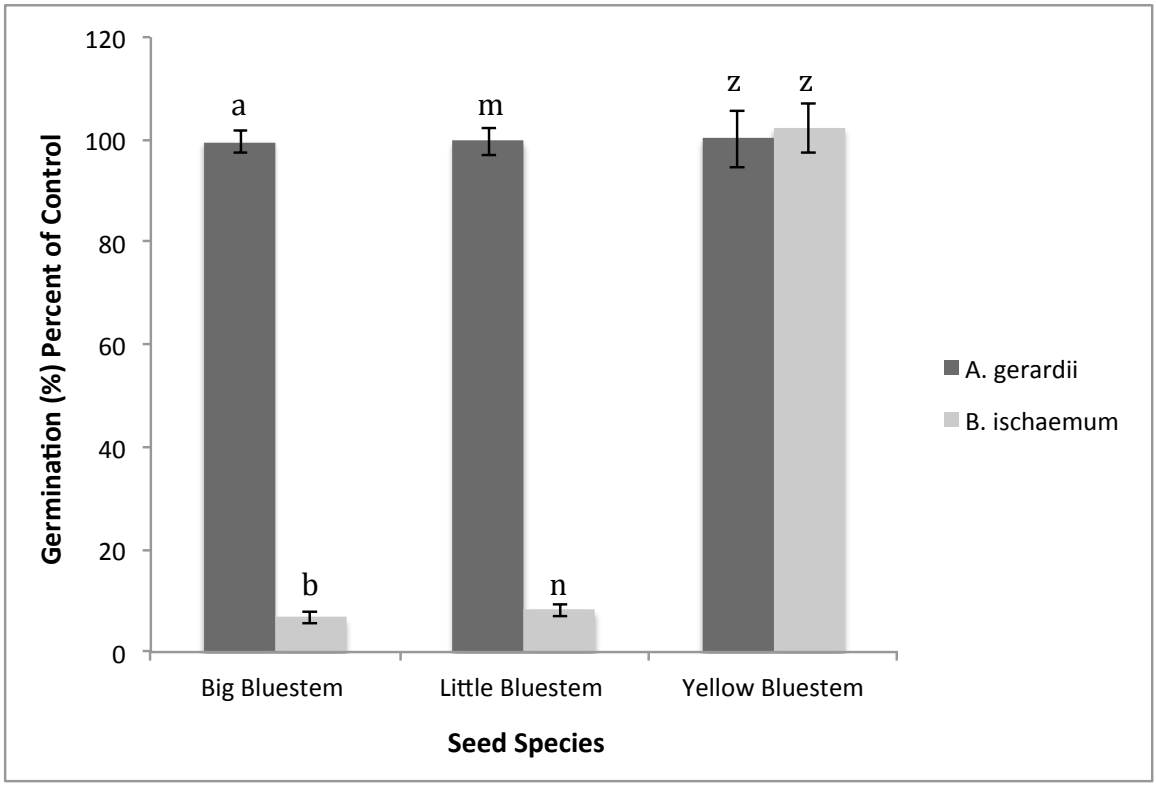
Treatment	Mean	Min.	Max.	SE
Control	265.08	235.67	280.00	14.71
A. gerardii Leachate	288.61	279.57	297.73	5.24
B. ischaemum Leachate	289.92	169.27	469.83	91.69
A. gerardii Leaf Litter	150.26	145.43	159.30	4.53
B. ischaemum Leaf Litter	209.04	173.17	277.67	34.23

Table 3. Mean, minimum, maximum, and standard errors values of pH, maximum absorbency, solubility, total phenols, and elemental composition (Na, Ca, Mg, K, S, B, P, Fe, Zn, Cu, Mn, and Al) determined for both *A. gerardii* and *B. ischaemum* leachates utilized during this study. Statistical differences (alpha = 0.05) between leachate types are denoted by bolded mean values, with corresponding t-statistics and p-values listed for each two-sample t-test.

Leachate Type	Variable	Mean	Min.	Max.	SE	t	p-value
A. gerardii	pH	5.36	5.08	5.67	0.17	0.91	0.417
B. ischaemum		5.77	5.32	6.62	0.42		
A. gerardii	Max Absorbance (nm)	288.67	280.00	282.00	0.67	1.00	0.423
B. ischaemum		280.00	280.00	280.00	0.00		
A. gerardii	Aqueous/Organic Solubility	Aqueous	NA	NA	NA	NA	NA
B. ischaemum		Aqueous	NA	NA	NA		
A. gerardii	Total Phenols (mg/l)	**642.08**	621.34	694.84	26.59	3.91	0.017
B. ischaemum		**265.16**	202.97	355.62	46.29		
A. gerardii	Na (mg/l)	3.50	2.42	4.92	0.74	1.23	0.340
B. ischaemum		9.73	3.72	19.71	5.02		
A. gerardii	Ca (mg/l)	175.04	128.71	248.88	37.32	0.21	0.845
B. ischaemum		191.38	55.47	276.83	68.70		
A. gerardii	Mg (mg/l)	78.90	62.32	104.29	12.89	0.15	0.890
B. ischaemum		83.92	29.27	138.14	31.43		
A. gerardii	K (mg/l)	603.78	577.11	652.96	24.62	1.22	0.291
B. ischaemum		458.19	248.38	653.53	117.2		
A. gerardii	S (mg/l)	45.01	36.63	55.88	5.70	0.51	0.638
B. ischaemum		50.96	36.99	70.93	10.25		

A. gerardii	B (mg/l)	0.22	0.22	0.23	0.01	1.26	0.332
B. ischaemum		0.17	0.11	0.25	0.04		
A. gerardii	P (mg/l)	19.88	17.45	24.63	2.37	0.09	0.934
B. ischaemum		19.17	4.53	29.88	7.58		
A. gerardii	Fe (mg/l)	4.37	1.95	5.64	1.21	0.24	0.820
B. ischaemum		4.93	1.53	8.29	1.95		
A. gerardii	Zn (mg/l)	0.60	0.45	0.76	0.09	1.82	0.142
B. ischaemum		0.33	0.21	0.57	0.12		
A. gerardii	Cu (mg/l)	0.03	0.02	0.03	0.01	0.81	0.461
B. ischaemum		0.02	0.01	0.03	0.001		
A. gerardii	Mn (mg/l)	3.24	2.92	3.78	0.27	0.88	0.435
B. ischaemum		3.89	2.52	4.82	0.70		
A. gerardii	Al (mg/l)	0.24	0.17	0.28	0.03	1.67	0.170
B. ischaemum		0.14	0.05	0.20	0.05		

FIGURES



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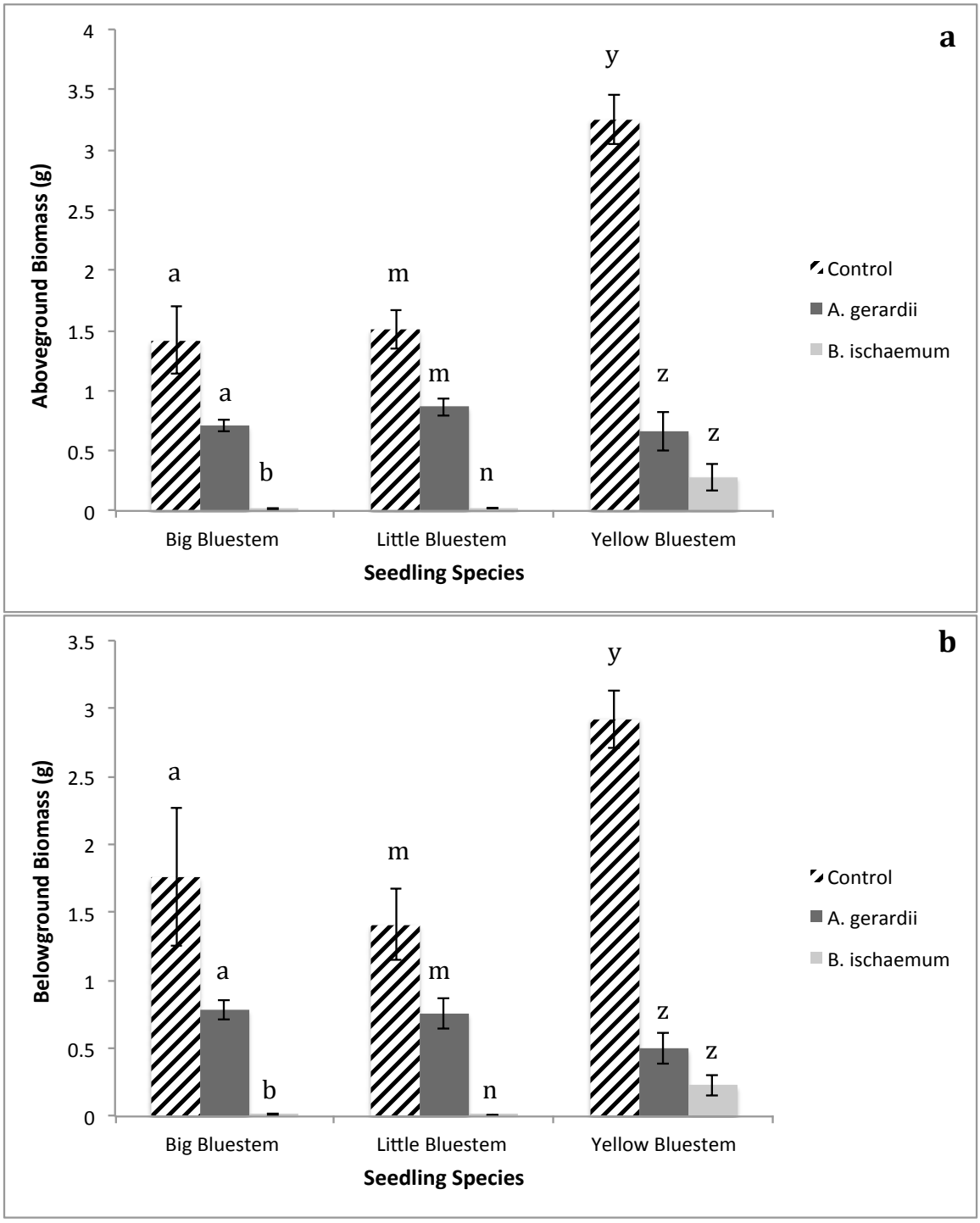
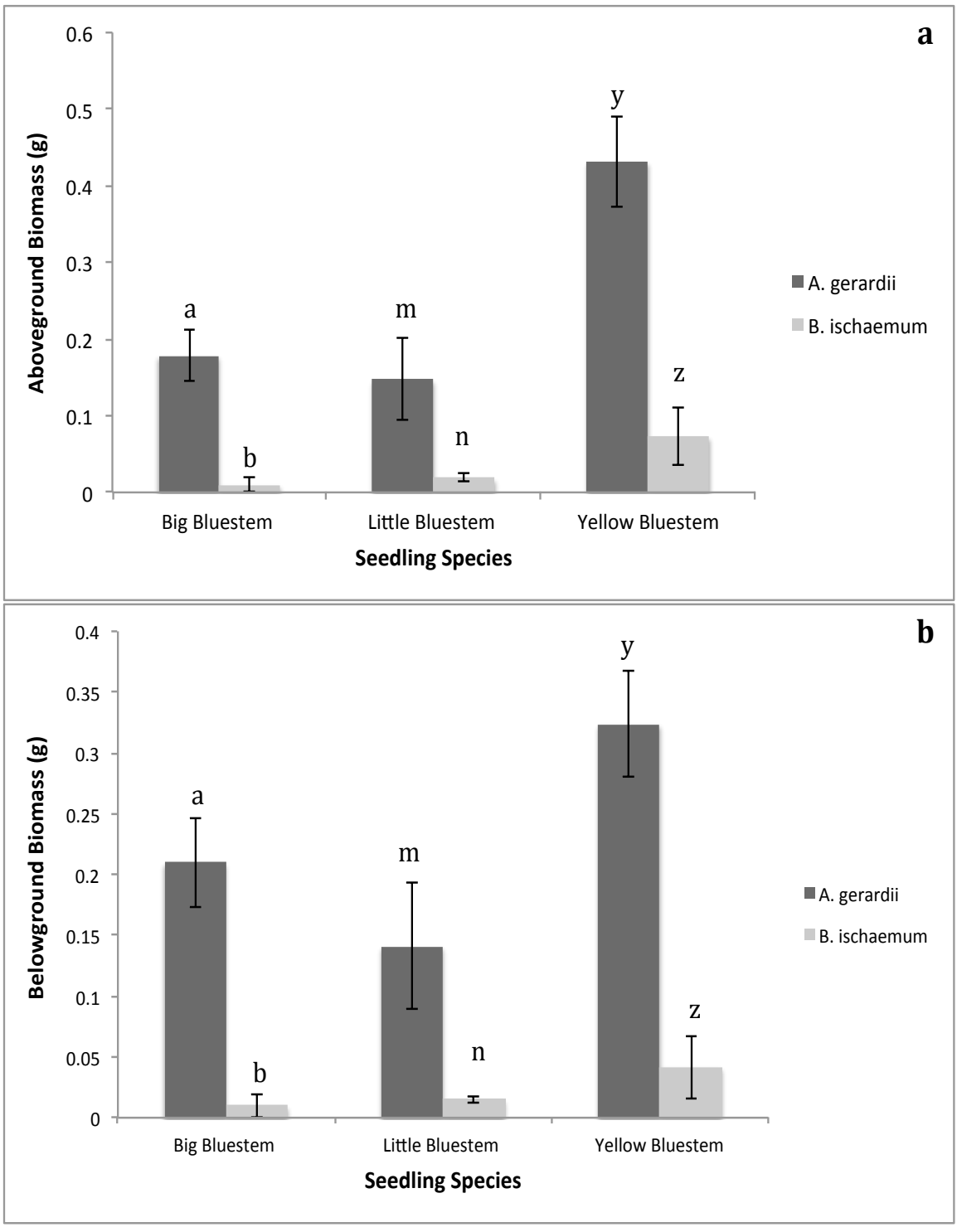

a
Aboveground Biomass (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
a
a
b
m
m
n
y
z
z
Big Bluestem
Little Bluestem
Yellow Bluestem
Seedling Species
Control
A. gerardii
B. ischaemum
b
Belowground Biomass (g)
3.5
3
2.5
2
1.5
1
0.5
0
a
a
b
m
m
n
y
z
z
Big Bluestem
Little Bluestem
Yellow Bluestem
Seedling Species
Control
A. gerardii
B. ischaemum

Figure 2.


a
Aboveground Biomass (g)
0.6
0.5
0.4
0.3
0.2
0.1
0
a
b
m
n
y
z
Big Bluestem
Little Bluestem
Yellow Bluestem
Seedling Species
A. gerardii
B. ischaemum
b
Belowground Biomass (g)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
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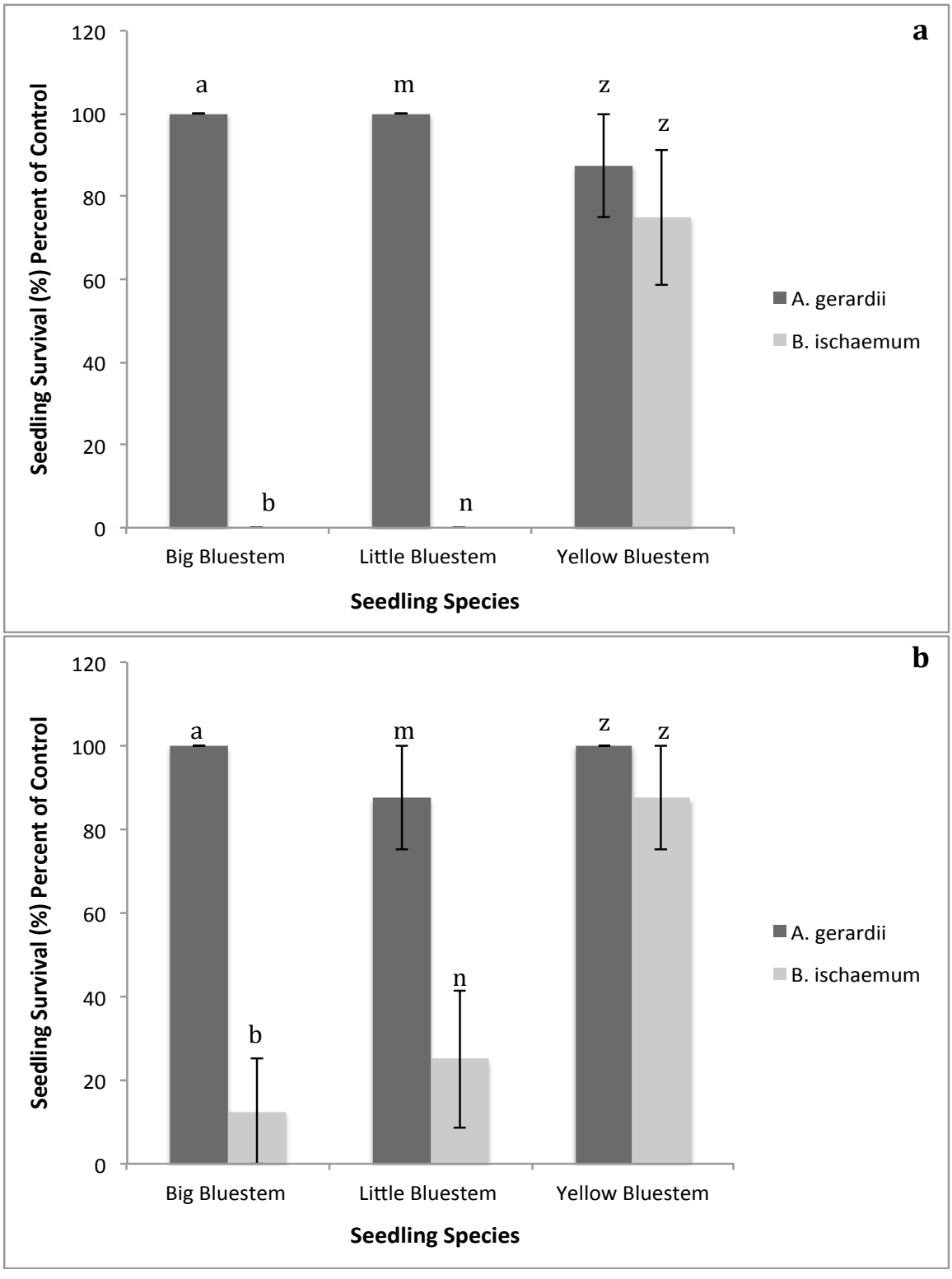
Big Bluestem
Little Bluestem
Yellow Bluestem
Seedling Species
A. gerardii
B. ischaemum

Figure 3.


a
120
100
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60
40
20
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Seedling Survival (%) Percent of Control
a
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Big Bluestem
Little Bluestem
Yellow Bluestem
Seedling Species
A. gerardii
B. ischaemum
b
120
100
80
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40
20
0
Seedling Survival (%) Percent of Control
a
b
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n
z
z
Big Bluestem
Little Bluestem
Yellow Bluestem
Seedling Species
A. gerardii
B. ischaemum

Figure 4.

FIGURE CAPTIONS

Figure 1: Seed germination reported as percent of the control for native grasses *Adropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), and invasive grass *Bothriochloa ischaemum* (yellow bluestem) after application of either *A. gerardii* or *B. ischaemum* leachate. Significant differences (alpha = 0.05) are indicated by different lower case letters, with comparisons made only within species not between species. Errors bars represent ±1 SE.

Figure 2: Aboveground (a) and belowground (b) biomass production for native grasses *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), and invasive grass *Bothriochloa ischaemum* (yellow bluestem) after application of dH$_2$O (control), *A. gerardii,* or *B. ischaemum* leachate. Significant differences (alpha = 0.05) are indicated by different lower case letters, with comparisons made only within species not between species. Errors bars represent ±1 SE.

Figure 3: Aboveground (a) and belowground (b) biomass production for native grasses *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), and invasive grass *Bothriochloa ischaemum* (yellow bluestem) after application of *A. gerardii* or *B. ischaemum* leaf litter. Significant differences (alpha = 0.05) are indicated by different lower case letters, with comparisons made only within species not between species. Errors bars represent ±1 SE.

Figure 4: Seedling survival reported as percent of the control for native grasses *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), and invasive grass *Bothriochloa ischaemum* (yellow bluestem) after application of either *A. gerardii* or *B. ischaemum* leachate (a) or leaf litter (b). Significant differences (alpha = 0.05) are indicated by different lower case letters, with comparisons made only within species not between species. Errors bars represent ±1 SE.

CHAPTER III

THE EFFECTS OF EXOTIC GRASS INVASION ON SMALL MAMMAL COMMUNITIES OF THE CENTRAL AND SOUTHERN GREAT PLAINS: AN ECOLOGICAL GAME CHANGER.

ABSTRACT

Bothriochloa spp. are invasive warm-season grasses that have been planted on millions of hectares in the southern and central Great Plains. These grasses are currently a major management concern due to their rapid invasion into native prairies, causing negative ecological and economical consequences, including possible alterations in small mammal communities. Previous studies have shown that as diverse native prairies give way to monocultures of invasive plants, wildlife diversity, richness, and abundances decline. We hypothesized that as invasions of exotic grasses progress towards monocultures, reductions in small mammal abundance and richness will occur. We assessed the effects of *Bothriochloa ischaemum* (yellow bluestem) invasions on small mammal communities in Oklahoma, USA. We conducted small mammal trapping at four replicate sites in grasslands with 40-60% *B. ischaemum* cover, and 4 replicate paired native, non-invaded grasslands. Multiple habitat metrics were assessed to allow for development of species-specific habitat models. We conducted 5,120 trap days and captured 193 individuals in native grasslands compared to 294 individuals in *B. ischaemum* invaded grasslands. Our data indicate that invasion of *B. ischaemum* into the native grasslands lowered all abundance

metrics of deer mice (*Peromyscus maniculatus*), while increasing all abundance metrics for hispid cotton rats (*Sigmodon hispidus*), as compared to native grassland controls. Species-specific models show cotton rats choosing vegetation that supplies aerial predator avoidance and deer mice selecting habitat that increases foraging efficiency. Because small mammals are vital to grassland ecosystems, alterations in these communities may have profound effects on ecosystem functioning.

INTRODUCTION

The largest percentage of global land cover is comprised of grasslands, savannahs, and other grass dominated systems, occupying up to half of the world's ice-free terrestrial landscape (Ellis and Ramankutty 2008). One of the most important grasslands types is the perennial native grassland. These grasslands supply many different ecological and economical goods and services across their multi-continental range. In North America, the Great Plains grassland ecosystem stretches approximately 3,200 km north to south from southern Canada to Texas and from the Rocky Mountains west approximately 800km. This 1,300,000 km^2 area provides many important ecological functions (e.g. wildlife habitat, CO_2 sequestration, addition of nutrients to the soil) but also produces numerous products utilized by humans (food, fuel, and fibers). The Great Plains ecoregion evolved as a grassland system with some areas developing fertile soil suited for agricultural practices such as grain production, while other areas of the central and southern Great Plains such as Oklahoma developed relatively nutrient poor soil that is highly erodible and are predominately used as rangelands (Redfearn 2007).

Currently the Great Plains are under "attack" by many different forces such as climate change, habitat fragmentation due to urbanization (e.g. roads, power lines), or habitat loss due to conversion to row crop agriculture. Nationwide, habitat loss totals 99.9% for tallgrass and 70-80% for mixed-grass prairies (Johnson 2006), leaving the grasslands of the Great Plains one of

the most endangered ecosystems in North America (Samson et al. 2004; Askins et al. 2007). Remaining blocks of native habitat in the Great Plains are also under "attack" by invasive plant species, which alter the structure of the local vegetation and degrades the quality of habitat in these prairie remnants (Scheiman et al. 2003). Many invasive species of the southern and central Great Plains are non-native grasses that have been purposely seeded onto deteriorated grasslands (McCoy et al. 1992; Harmoney et al. 2007). However, the rapid spread of these grass species into native grasslands is causing ecological and economic concerns (Harmoney and Hickman 2004).

A group of warm-season invasive grasses, collectively known as Old World Bluestems contains numerous genera with *Bothriochloa spp*. being most problematic in the central and southern Great Plains. *Bothriochloa spp.* have been introduced from numerous areas including Europe, Asia, Russia, and Australia and are capable of producing four times as much forage as native species in well managed grasslands (McCoy et al.1992; Schmidt et al. 2008). It is estimated that *Bothriochloa spp.* have been planted onto millions of hectares of marginal farmland, roadside right-of-ways, and Conservation Reserve Program (CRP) lands in the central and southern Great Plains (White and Dewald 1996; Harmoney and Hickman 2004). Although beneficial as forage, *Bothriochloa spp.* need to be intensely managed and harvested at appropriate times as forage quality and grazer preference decline rapidly as the plant matures (Dabo et al. 1988; Briske 1991; Harmoney and Hickman 2004; Burns 2011). There are also ecological concerns as *Bothriochloa spp.* often escape original planting sites and invade native grasslands (Reed et al. 2005; Baer et al 2009). Reed et al. (2005) described *Bothriochloa spp.* as creating moving "fronts" of invasion into native warm-season dominated grasslands in Kansas. Research by Schmidt et al. (2008) and Wilson et al. (2012) have shown that *Bothriochloa spp.* are better competitors than native warm-season grass species. When *Bothriochloa spp.* and native warm-season grasses were planted together, the native grasses were unable to grow beyond the seedling stage, while *Bothriochloa spp.* produced greater biomass than when paired with conspecifics

(Schmidt et al. 2008; Wilson et al. 2012). This competitive advantage allows *Bothriochloa spp.* to create large monospecific stands where native grasslands rich in diversity once stood.

The loss of plant biodiversity can alter soil nutrient and texture (Ruffner 2012), with cascading effects on ecosystem function through upper trophic levels. Grasslands comprised of monospecific stands of invasive grass species have been shown to have lower bird diversity (Bakker and Higgins 2009) and vegetative productivity (Lloyd and Martin 2005). Hickman et al. (2006) reported lower arthropod biomass, lower bird species richness, and lower individual bird species abundance in *Bothriochloa spp.* dominated fields, as compared to native grasslands. Similar studies have reported declines or alterations in small mammal diversity, richness, and relative abundance as grasslands progress from native vegetation to exotic grass-dominated sites (Schwartz and Whitson 1987; Hayslett and Danielson 1994; Spyreas et al. 2010; Litt and Steidl 2011). Small mammals are a vital component of grassland ecosystems; they collect and distribute seeds (Wilson 1993) and fungal spores (Maser et al. 1978; Gehring et al. 2002) through foraging activities and they represent the lower trophic level and food source for upper level carnivores inhabiting grasslands. An increase or decrease small mammal species can have profound effects on the ecosystem and its functioning. It has been suggested that lower abundance and diversity of birds, small mammals, and arthropods in grasslands dominated by *Bothriochloa spp.* is due to the growth patterns of the invasive grasses, promoting the development of monospecific stands with fewer microhabitats and resource bases than highly diverse native grasslands (Sammon and Wilkins 2005; Hickman et al 2006; Bakker and Higgins 2009).

To assess species richness and relative abundances of small mammal communities of *Bothriochloa ischaemum* (L.) Keng invaded and native grasslands of the southern and central Great Plains we conducted a two year field study in Payne and Noble Counties OK, USA. Based on previous research (Sammon and Wilkins 2005; Hickman et al. 2006; Spyreas et al. 2010; Litt and Steidl 2011) we hypothesized that species richness of the small mammal community would

be lower in *B. ischaemum* invaded grasslands, as compared to that of native prairie, due to loss of microhabitats and resource bases as diverse native prairies become invaded and progress towards monospecific stands of *B. ischaemum*. We further hypothesized that small mammal communities of the invaded sites will be dominated by a greater abundance of few species capable of occupying *B. ischaemum* invaded grasslands, as compared to more diverse communities of the native sites. Understanding the consequences of *Bothriochloa spp.* invasion on ecosystem function of native grasslands is essential to accomplishing successful restoration of invaded sites following eradication of the exotic plant species.

MATERIAL AND METHODS

Site description

We surveyed small mammal communities of Payne and Noble Counties in north central Oklahoma. Our sites were located approximately 12 km west of Stillwater, Oklahoma, USA. This area is located in the western edge of the Cross Timbers ecosystem which occurs from southeastern Kansas to north central Texas. The Cross Timbers ecosystem is a mosaic of upland deciduous forest, savanna, and tallgrass prairie that forms a transition zone between the eastern deciduous forest and the grasslands of the southern Great Plains (Van Els et al. 2010). Average temperatures for the region vary between seasons with summer temperatures averaging 34.0° C and winter temperatures averaging -1.0° C. Annual precipitation averages 93.2 cm with a peak in rainfall normally occurring in May. The soils of this area tend to be loam-dominated with pockets of fine sandy loam and silty loam (USDA NRCS 2007). Primary landuse of this area is rangeland for cattle production with small pockets of farmland primarily growing wheat (*Triticum astevium* [L.]) and corn (*Zea maise* [L.]).

We selected grasslands with sites large enough to encompass our transects and corresponding buffers (minimum of 16.5 ha). Four native prairie sites and four *B. ischaemum*

invaded sites were randomly selected from available lands meeting the above criteria. All *B. ischaemum* sites were in the process of invading adjacent native grasslands; none had established monospecific stands, but each contained 40-60% aerial coverage of *B. ischaemum*. Native sites were typical of the central and southern Great Plains and were dominated by warm-season grasses such as big bluestem (*Andropogon gerardii* [Vitman]) little bluestem (*Schizachyrium scoparium* [(Michx.) Nash]), switchgrass (*Panicum virgatum* [L.]), and indiangrass (*Sorghastrum nutans* [(L.) Nash]) and a variety of forbs and shrubs. The grassland sites we selected were under similar cow/calf herd management practices with rotational grazing periods ranging from 2-4 weeks followed by a minimum of 2 months rest in between periods and a moderate stocking rate. All pastures were periodically burned in the spring with 2-4 year fire return intervals.

Small mammal habitat measurements

To evalaute small mammal structure and composition eof *B. ischaemum* invaded sites and native grasland sites, we evaluated multiple local habitat characteristics (Table 1). To reduce the influence of habitat differences when comparing small mammal communities between native and *B. ischaemum.* invaded sites, we surveyed vegetation twice each year (June: peak production time for cool-season and November: peak production time for warmseson grasses in this region) (Phelps and McBee 2009). Vegetation measurements were collected within 1 m of the 1st, 5th, 10th, 15th, and 20th trap location of each transect (Sammon and Wilkins 2005). Visual obstruction was measured using a Robel pole placed directly on the transect line. A measurement was taken in each of the four cardinal directions at a height of one meter, a distance of four meters, and to an obstruction of 100% and recorded to the nearest 0.25 dm (Robel et. al. 1970). Litter depth was measured at the four points corresponding to Robel measurements using a millimeter ruler placed into the litter until it made contact with the soil and then read to the nearest millimeter. A 1 m^2 frame was used to estimate percent cover using the Daubenmire cover classes for total grass, C_3 species, C_4 species, forbs, litter, bare ground, "other" (e.g. rocks, manure), woody plants, standing

dead material, and native plant species vs. *B. ischaemum* at every survey station (Daubenmire 1959). To assess the cover (aerial predator avoidance) provided by each vegetation class, we measured total grass, forb, standing dead material, native species, and *B. ischaemum* using the Daubenmire cover class method and elevating our 1 m^2 frame to multiple heights (5 cm, 10 cm, 25 cm, 50 cm) above the soil (Sammon and Wilkins 2005). The midpoint value was used for data analysis of all cover class estimates.

Small mammal trapping

Within each site we randomly established a transect of 20 Sherman (7.6 x 8.9 x 22.9 cm; H. B. Sherman Traps, Inc., Tallahassee, Florida) live traps placed in a straight line at 15m intervals with a single trap placed at each location (Rehmeier et al. 2005). In large fields, a second transect was included to standardize sampling effort per site. The two transects were averaged together before analysis. Straight line transects were selected as previous studies have indicated these are more effective for accurately determining species composition then trapping grids (Pearson and Ruggiero 2003). Transects were placed ≥50 m away from any edge (road, wetland, shelter belt, or field of differing management) to decrease the probability of capturing non-grassland species and avoid the influence these edges may have on species richness and relative abundance (Perry and Thill 2005). Each trap was baited with a mixture of peanut butter and oatmeal (Rehmeierr et al. 2005); cotton nesting material was placed in the trap during cold periods (November and February) to prevent hypothermia (Gannon and Sikes 2007). We checked traps a minimum of twice daily (24 hr period; just after dawn and just before dusk) but increased daytime checks as ambient temperatures increased (August), to help minimize capture stress and trap mortality (Côté and Ferron 2001). Trapping was conducted for two years (2010-2012), with four season per year (February, May, August, and November), with traps being open for four consecutive 24 hr periods per season (Sammon and Wilkins 2005). We trapped all sites (native

and *B. ischaemum* invaded) at one time to reduce variability in stochastic events between trapping sets in one season.

Each captured individual was weighed to the nearest gram, identified to species, sexed, and aged (juvenile or adult). Each individual was marked with a felt tipped permanent marker on the underside of their jaw and neck to allow individuals recaptured during a single season to be easily identified. Marking in this location prevented the animal from grooming the area where the mark is placed and removing the mark. Recaptured individuals were recorded but not used in analysis. All specimens were released at the capture site directly after data were recorded. All capture, handling, and marking procedures were conducted following the standards set forth by the Animal Care and Use Committee of the American Society of Mammalogist (1998), under Oklahoma State University Animal Care and Use Protocol #AG-10-5 and followed the recommendations of Gannon and Sikes (2007).

Statistical analysis

For each species at each transect, we determined an index of abundance defined as number of captures of a given species divided by the total number of trap nights multiplied by 100 (Schwartz and Whitson 1987; Hanchey and Wilkins 1998; Jenks et al. 2011). Relative abundance was defined as the number of individuals of a given species divided by the total number of individuals of all species captured (Nolte and Fulbright 1997). Species richness (total number of species caught) was also calculated for each transect (Sammon and Wilkins 2005). Seasonal variability is known to exist for many species of small mammals (Kaufman et al. 1995, Matlack et al. 2002), and since our objective was to evaluate habitat of the entire small mammal community we combined data from all four trapping season within a single year. To determine if differences between years (2011 vs. 2012) existed within habitat types (native or *B. ishaemum* invaded) for habitat variables and/or small mammal capture metrics we conducted two-sample t-

tests at an alpha = 0.05 (Freund and Wilson 2003). Differences in habitat characteristics between years were minimal and are reported in the results to illustrate the impacts of drought during our study. However, since few of the assessed habitat variables differed between years, we combined data before determining differences between habitat types (native vs. *B. ischaemum* invaded). Two-sample t-tests were used to determine if differences existed between native and *B. ischaemum* invaded sites for all habitat variables at alpha = 0.05 (Freund and Wilson 2003). For model development habitat measurements were paired with trapping seasons that corresponded to C_3 and C_4 species dominace in our study area (i.e. June vegetation samples were paired with February and May trapping seasons and November vegetation samples were paired with August and Novemeber trapping seasons). Between year differences did not exist for any of the small mammal capture metrics, therefore years were combined before determining average transect values for further analysis. Two-sample t-tests were used to determine if differences existed between the native and *B. ischaemum* invaded sites for all small mammal community metrics at alpha = 0.05 (Freund and Wilson 2003).

To assess local area variables and the potential for habitat effects on small mammal abundances at each transect we used "*a priori*" models established from the literature and conducted linear regressions to model habitat variable influence for all species with 30 or more captures (Hosmer and Lemeshow 1989; Kutner et al. 2004). To prevent issues with multicollinearity, variables were only included in models if they were not correlated ($|r| < 0.5$) with variables already in the model (Fletcher and Koford 2002). We used Akaike's information criterion corrected for small sample size (AICc) as a basis for model selection (Akaike 1969; Liberton et. al. 1992; Burnham and Anderson 1998). Models that yielded the smallest AICc value were considered the most parsimonious and the best approximation for the information in the data set (Burnham and Anderson 1998). Burnham and Anderson (1998) suggest reporting the change in AICc (ΔAICc) instead of the AICc value itself since it is the relative change in AICc between

models that is important to show support for developed models. In all tables comparing any developed models we report AICc, ΔAICc, and the Akaike weights (AICω). Akaike weights indicate the probability that a given model is the expected best model of all candidate models (Akaike 1978, 1980 and 1983; Anderson 2008). We considered any model with a AICω of ≥0.01, when compared to the "best" model after removal of "pretending" vaiables to be a competitive alternative.

Model selection using AICc can result in the selection of overly complex models by including uninformative or "pretending" variables (Richards 2008; Arnold 2010). These "pretending" variables appear in models that have simpler models nested within them resulting in only a slight increase in AICc, but do not increase the fit of the model according to its maximum likelihood (Richards 2008; Arnold 2010). Arnold (2010) offers five options for dealing with these "pretending" variables; we have selected the option to remove models that contain "pretending" variables and re-conduct the analysis as is also advocated by Richards (2008). This option was selected for our data, as we were interested in constructing a few "best" models resulting in determination of the most pertinent variables and avoiding models with erroneous support. All analyses were completed using Program R (R Core Team 2012), MASS (Venables and Ripley 2002), and pscl (Zeileis et al. 2008; Jackman 2012) packages for basic calculations, and the emdbook (Bolker 2012) and bbmle package for AICc and Akaike weights calculations (Bolker and R Core Team 2012).

RESULTS

Vegetative characteristics

Vegetative habitat characteristics were compared between the two years of trapping (2011 and 2012). Native sites showed few significant differences between years. Amount of forb coverage, native plant coverage, and native plant coverage 5 cm above ground level decreased

between year one to year two, while the amount of litter coverage and amount of standing dead material at the 0 cm, 5 cm, and 10 cm measurements increased in this same time period (Fig. 1). *B. ischaemum* invaded sites also had few significant differences between years in any parameters we measured. Percent cover of standing dead at 0 cm, 5 cm, and 10 cm above soil increased from year 1 to year 2, while amount of total grass coverage at 5 cm and 10 cm decreased (Fig. 2).

Because few vegetative habitat characteristics differed between years, we combined years to determine an overall average for each variable (Fig. 3). Analyses of habitat indicated only three variables were significantly different between the native and *B. ischaemum* invaded grasslands. Native grassland sites had significantly greater cover of native plant species (at 0 cm, 5 cm, and 10 cm above soil) compared to *B. ischaemum* invaded sites. However, *B. ischaemum* invaded sites had significantly greater *B. ischaemum* percent cover at 0 cm, 5 cm, 10 cm, and 25 cm above soil surface (Fig. 3). Litter depth was visibly and also significantly different between habitats, with *B. ischaemum* habitats having greater litter depth than native sites (Fig. 3).

Small mammal captures and indices

Total trapping effort for this study was 5,120 trapping days with each day consisting of a 24 hr period. The total number of captures was 487 individuals across all transects and seasons and consisted of 8 species (Table 2). We captured more individuals in *B. ischaemum* invaded transects (n=294) than native transects (n=193). Cotton rats (*Sigmodon hispidus*) were the dominant species captured overall (n=311) and the most dominant species observed in *B. ischaemum* dominated grasslands (n=237) (Table 2). Deer mice (*Peromyscus maniculatus*) were the second most abundant species captured overall (n=137) and were the most abundant species captured in native sites (n=101) (Table 2). When comparing the raw abundance (Fig. 4a), abundance index (Fig. 4b), and relative abundance (Fig. 4c) of all 8 species, we found that only deer mice showed significant differences between the native and *B. ischaemum* invaded

grasslands with natives sites having higher values for all deer mice metrics relative to *B. ischaemum* invaded sites. We also found that species richness of small mammals was not significantly different between the two habitat types.

Habitat models

After removal of models containing pretending variables, we had three candidate models for cotton rats abundance and 11 models for deer mice abundance (Table 3). AICc selected "best" models for cotton rat and deer mice have Akaike weights of 0.8942 and 0.3034 indicating the "best" models have probabilities of 89.42% and 30.34% respectively, for being the best model of all candidate models (Table 3). The "best" model for cotton rats indicated that their abundance was negatively associated with bareground and positively with aerial coverage supplied by *B. ischaemum* at 50 cm above ground level (Table 3). Other "top" models indicated that cotton rat abundance was also positively associated with amount of aerial coverage provided by all grass species at a height of 50 cm within the grassland (Table 3). Deer mice abundance responded to a larger suite of variables than cotton rats. The "best" model for deer mice indicated that their abundance was negatively associated with amount of total grass coverage present in the grassland (Table 3). Deer mice abundance was also found to be negatively associated with amount of *B. ischaemum* coverage, amount of C_4 species coverage, litter depth, visual obstruction, and aerial coverage supplied by *B. ischaemum* at heights of 5 cm, 10 cm, and 25 cm above ground level, and aerial coverage supplied by all grass species at 5 cm above soil surface (Table 3). Deer mice were positively associated with amount (not depth) of litter coverage in these grasslands (Table 3).

DISCUSSION

Our results indicate that *B. ischaemum* invasion has potential to alter the habitat structure as it invades native grasslands in the central and southern Great Plains. These alterations to habitat structure correspond to converse alterations in small mammal communities that inhabit

these grasslands. These alterations are most likely related to the foraging habits and predator avoidance behavior of the small mammals.

These alterations are of ecological concern as invasion by invasive plant species has increased at unprecedented rates due to human activities that have amplified the number of introductions and the rate of spread for many exotic species (Chapin et al. 2000). Tremendous economical impacts are associated with biological invasions (Vitousek et al. 1997; Pimentel et al. 2000), through the cost of control or restoration of invaded sites or as direct loss or reduction of product (such as sustainable livestock production). Pimentel et al. (2005) estimated the cost of control and lost or damaged goods due to all exotic species in the US to be ~120 billion dollars, with exotic plants comprising ~35 billion dollars (and entailing at least 25,000 plant species). Additionally, plant invasions result in loss of ecological services. Exotic plant invasions bring about changes in intensity and frequency of disturbance regimes (D'Antonio and Vitousek 1992), alter ecological structure and functioning (Chapin et al. 2000), and ultimately alter native species biodiversity (plants and wildlife) (Heywood 1989). These alterations in ecosystem function following biological invasion by exotic plant species has been viewed as one of the major current causes of native grasslands loss (Watkinson and Ormerod 2001) and global change (Vitousek et al. 1997).

There are also numerous studies that have determined exotic plant invasions result in bottom-up alterations though modifying habitat structure and resource bases, leading to alterations in consumer behavior (Wolkovivh et al. 2009; Dutra et al. 2011). These bottom-up alterations to biodiversity result when invasive plant species create positive feedback loops, allowing the production of monospecific stands of exotic species, with a concomitant loss of native plant species (Bever 2003). The replacement of diverse native habitats by monospecific stands of invasive plant species has been documented to alter wildlife biodiversity of native grasslands. Hickman et al. (2006) showed that *Bothriochloa spp.* monocultures contained lower

arthropod biomass than native prairie remnants, which directly related to lower grassland bird species richness and abundance in *Bothriochloa spp.* monocultures. Studies have also shown negative effects of monocultures of exotic grasses, including monocultures of King Ranch Bluestem (*B. ischaemum*), on small mammal communities of native prairies (Schwartz and Whitson 1987; Hayslett and Danielson 1994; Sammon and Wilkins 2005).

Monospecific stands of vegetation lack the heterogeneity necessary to create microhabitats and food resources required to support diverse small mammal communities. However, to date there has not been documentation that small mammal communities of native grasslands are negatively affected by expanding populations of exotic plants. However, understanding potential effects throughout various stages of exotic grass expansion is essential for successful restoration of native grasslands. Small mammal response to invasion by exotic plants may lag behind the alterations of the vegetative structure, which indicates that there may be benefit to eradicating *B. ischaemum* before it reaches a monospecific stand as once near monospecific stands are reached the small mammal community may begin to shift. Therefore, in this study we selected native prairies currently being invaded by *B. ischaemum*, with our invaded sites containing 40-60% *B. ischaemum* cover (i.e. not monospecific stands).

Between year (2011 and 2012) comparisons of vegetative structure between the native and *B. ischaemum* invaded sites indicated few differences. However, both native and *B. ischaemum* invaded sites experienced reductions in vegetation cover in 2012, as compared to 2011. Native sites exhibited reductions in percent of native grass and forb cover from soil to 5 cm above ground, and *B. ischaemum* invaded sites exhibited reductions in percent grass cover of *B. ischaemum* and native grasses at both 5 cm and 10 cm above the soil surface. These differences are likely related to the intensive drought experienced in the summer of 2012. Total summer (May-August) precipitation for 2011 was 16.64 cm, 42% lower than the average rainfall (1971-2000). However, summer (May-August) precipitation of 2012 was substantially lower than

normal with only 24.19 cm of rainfall, a 61% decrease from average precipitation (1971-2000) (Mesonet data; Brock et al. 1995). Reductions in vegetative cover are not unexpected during periods of low precipitation, as summer drought has been well documented to reduce aboveground production in grassland systems, both in the current growing season and with possible legacy effects for future growing seasons (Bloor et al. 2010; Cantarel et al. 2013). Both native and invaded habitats experienced increased standing dead material at both 5 cm and10 cm above soil surface in 2012, as compared to standing dead recorded in 2011, presumably due to greater plant death as drought intensity increased. Comparison of plant species communities between the native and invaded habitats resulted in several significant differences. Native grasslands had greater percent native plant cover at each measured distance above soil surface; 0 cm, 5 cm, and 10 cm, compared to *B. ischaemum* invaded sites. Not surprisingly, *B. ischaemum* invaded sites had greater cover of *B. ischaemum* at most gradients above soil level; 0 cm, 5 cm, and 10 cm, and 25 cm, as compared to native grasslands. Litter depth was also different between the two habitats, with *B. ischaemum* invaded grasslands characterized by greater litter depths than native grasslands. These differences in plant community structure were easily observed in the field, as were differences in growth form of the native and exotic grasses. Native species produced fewer, but taller, stems while *B. ischaemum* produced numerous short stems, resulting in increased percent total grass cover at the 25 cm and 50 cm measurements in *B. ischaemum* invaded sites, compared to percent total grass cover of the native sites, at these same distances from the soil surface. At heights of 25 and 50 cm above the soil surface, native grass cover is composed of only a few stems while *B. ischaemum* has multiple stems, leaves, and seed heads. Differences in growth between native and *B. ischaemum* were not observed at distances closer to the ground as growth form at this height of both species is similar. This difference in grass structure allows for greater cover, with a concomitant increase in aerial predator avoidance. *Bothriochloa spp*. have been documented to be superior competitors when compared to native grass species, producing greater amounts of both below- and aboveground biomass (Schmidt et

al. 2008). This increased biomass results in greater litter production and thus greater litter depth. This positive correlation between *B. ischaemum* biomass and litter depth was apparent in our sites when assessing variable multicollinearity, as litter depth was positively correlated ($|r| > 0.5$ or greater) with all *B. ischaemum* cover variables.

These differences in habitat composition may have led to the differences in small mammal communities. All small mammal abundance metrics (raw abundance, abundance metric, relative abundance) measured for cotton rat indicated there were no differences in abundance between native and invaded sites. However, a prominent trend of greater cotton rat abundance in *B. ischaemum* dominated grasslands was apparent. Cotton rat captures did not significantly differ between years; however capture rates declined for all habitats in 2012. This decrease in cotton rat abundance may have played a vital role in the lack of significance between cotton rat abundances between the native and invaded sites, as these mammals have been reported to select *B. ischaemum* monocultures over native prairie (Sammon and Wilkins 2005). The reduction in cotton rat captures during 2012 may be attributed to the increased drought during our trapping seasons, as spring and summer droughts may lower reproductive success of this species. Cotton rats have an annual bimodal population increase (spring and fall) and summer droughts may prevent fall population increases of this species (Odum 1955). It is also possible that the lack of significant differences in cotton rat abundance metrics is related to the vegetative composition of our study sites as our fields of 40-60% *B. ischaemum* invasion do not posses the same structure as monocultures. As our study sites push towards monocultures, we may see a significant increase in cotton rat abundances. Our models for cotton rat abundance show trends similar to previous studies, with greater cotton rat abundance being associated with variables that increase aerial predator avoidance, including litter depth (Fleharty and Mares 1973; Wilkins 1995), as aerial predators are a major cause of cotton rat mortality (Wiegert 1972).

Deer mice abundance was greater in native sites, as compared to *B. ischaemum* invaded sites, for both years. Again, these differences appear to be related to structural differences in the vegetation, as native sites were characterized by greater cover of native grasses, and native grasses produced less biomass, litter depth, and cover, as compared to *B. ischaemum*. Our models are concurrent with previous literature showing that deer mice prefer grasslands with less vegetation cover and lower litter depths (i.e. more open habitats) (Kaufman et al. 1990; Wilkins 1995; Kantak 1996; Pearson et al. 2001) including grasslands invaded by *B. ischaemum* in our study. Our models indicate deer mice are limited more by foraging capability than aerial predator avoidance. In support of our results, Clark et al. (1991) showed that deer mice recovered smaller amounts of seed and had lower seed foraging efficiency as litter depth increased while Clark and Kaufman (1991) reported that deer mice tended to nest and forage in habitats with sparse vegetation and minimal litter depths. It is also possible that the habitat selection differences of deer mice and cotton rats that were observed in our study is a reflection of foraging abilities of both species, due to body size and morphological differences (Clark and Kaufman 1991). The larger body size of cotton rats allows for foraging in dense vegetation and deep litter depths of *B. ischaemum* invaded sites unavailable to the smaller deer mice.

It has been previously documented that land management practices, such as grazing and fire, also play a vital and influential role in the habitat structure of grasslands across the Great Plains. Our habitats were selected to minimized the variation in land management between transects and treatments. However, fire history differed slightly as the native sites had been burned the year prior to our study (2010), while the invaded sites had not been burned for 3 years (2008). Previous literature has reported deer mice are often the dominant small mammal species in recently burned habitats, having abundances as much as 10 times greater than sites that had not been burned for three years (Fuhlendorf et al. 2010; Kirchner 2011). Cotton rat abundances of our study also agree with previous literature, with significantly lower cotton rat abundances in

recently burned grasslands. However, differences between our sites may be transitional, as cotton rat abundances began to increase just 8 months post fire (Kirchner et al. 2011) and became the dominant mammal species as quickly as 2 years post fire (Fuhlendorf et al. 2010). Fire may alter vegetative structure to a greater extent than it alters vegetative composition in these grasslands. *Bothriochloa spp.* has been reported to produce greater amounts of biomass (Schmidt et al. 2008) and litter depth, compared to native grasses. Therefore, similar fire return intervals of *Bothriochloa spp.* invaded sites may result in greater biomass and litter, compared to native grass dominated sites. Therefore, fire management of *Bothriochloa spp.* invaded grasslands may require more frequent fires than historically occurred to maintain the habitat structure and small mammal communities to that of native grasslands. However, this increased fire return interval would not be without ecological consequence. Reed et al. (2005) suggests that *Bothriochloa spp.* have a slightly higher C:N ratio than natives, and are capable of producing much greater biomass. This may lead to more nitrogen lost through volatilization during prescribed burns. This reduction in nitrogen could lead to deficits too great for native species to survive, although these native grasses are adapted to low nitrogen soils and have relatively high nitrogen use efficiencies. In this way, the invasive grasses may "beat the natives at their own game" (Reed et al. 2005). These low nitrogen levels may create positive feedback loops allowing for further invasion and development of *Bothriochloa spp.* monocultures, with consequential negative effects on biodiversity.

Invasive plant species, especially those capable of self-facilitation such as *Bothriochloa spp.*, are a global problem and no simple solutions have been proposed for their control or eradication. Our current research indicates invasive plant species are capable of altering biodiversity both directly and indirectly through both bottom-up and top-down effects. Although we observed only a few differences in the abundances of the species, the differences we did observe indicate that a shift in the small mammal community is occurring as *B. ischaemum* invasion increases. Our study sites were not monocultures of *B. ischaemum* but rather were in the

process of invasion and the lack of significant small mammal community shifts may be due to the lag response time of the small mammal community to the altered habitat structure. Our results indicate that alterations of the small mammal communities may be mitigated if control of *B. ischaemum* occurs before it reaches 60% cover. However, caution must be used in selecting control/management techniques as management through increased fire frequency may help to alleviate the changes in habitat structure but may come with greater ecological consequences.

REFERENCES

Akaike H. (1969) Fitting autoregressive models for prediction. Ann I Stat Math 21:243-247

Akaike H (1978) A Bayesian analysis of the minimum AIC procedure. Ann I Stat Math 30:9-14

Akaike H (1980) Likelihood and the Bayes procedure (with discussion). In: Bernardo JM, De Groot MH, Lindley DV, Smith AFM (eds) Bayesian statistics. University Press, Valencia, Spain, pp143-203

Akaike H (1983) Information measures and model selection. Int Stat I 44:277-291

Anderson DR (2008) Model based inferences in the life sciences. Springer Science and Business Media, LLC, New York, NY

American Society of Mammalogist, Animal Care and Use Committee (1998) Guidelines for the capture, handling and care of mammals as aproved by The American Society of Mammalogist. J Mammal 79:1416-1431

Arnold TW (2010) Uninformative parameters and model selection using Akaike's information criteria. J Wildlife Manage 74:1175-1178

Askins RA, Chavez-Ramirez F, Dale BC, Haas CA, Herkert JR, Knopf FL, Vickery PD (2007) Conservation of grassland birds in North America: understanding ecological processes in different regions. Ornithol Monogr no. 64:1-46

Baer SG, Engle DM, Knops JMH, Langeland KA, Maxwell BD, Menalled FD, Symstand AJ (2009) Vulnerability of Rehabilitated Agricultural Production Systems to Invasion by Nontarget Plant Species. Environ Manage 43:189-196

Bakker KK, Higgins KF (2009) Planted grasslands and native sod prairie: equivalent habitat for grassland birds? West N Am Naturalist 69:235-242

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol 157:465-473

Bloor JMG, Pichon P, Falcimagne R, Leadley P, Soussana JF (2010) Effects of warming, summer drought, and CO_2 enrichment on aboveground biomass production flowering phenology and community structure in an upland grassland system. Ecosystems 13:888-900

Bolker B (2012) emdbook: Ecological Models and Data in RR package version 1.3.2.1

Bolker B, R Development Core Team (2012) bbmle: Tools for general maximum likelihood estimation. R package version1.0.5.2

Briske DD (1991) Developmental morphology and physiology of grasses.. In: Heitschmidt RK, Stuth JW (eds) Grazing management: An ecological perspective. Timber Press, Portland, OR pp 85–108

Brock FV, Crawford KC, Elliot KC, Cuperus RL, Stadler SJ, Johnson HL, Eilts MD (1995) The Oklahoma Mesonet: a technical overview. J Atmos Oceanic Tech 12:5-19

Burnham KP, Anderson DR (1998) Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York, NY

Burns JC (2011) Maturity and regrowth influences on quality of Caucasian bluestem hay. Crop Sci 51:1840-1849

Cantarel AAM, Bloor JMG, Soussana JF (2013) Four years of simulated climate cange reduce aboveground productivity and alters functional diversity in grassland ecosystem. J Veg Sci 24:113-126

Chapin FS III, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU (2000) Consequences of changing biodiversity. Nature 405:234-242

Clark BK, Clark BS, Jacobi EA (1991) Ability of prairie rodents to find seeds in plant litter. Am Midl Nat 126:385-391

Clark BK, Kaufman DW (1991) Effects of plant litter on foraging and nesting behavior of prairie rodents. J Mammal 72:502-512

Côté M, Ferron J (2001) Short-term use of different residual forest structures by three sciurid species in a clear-cut boreal landscape. Can J Forest Res 31:1805-1815

Dabo SM, Taliaferro CM, Coleman SW, Horn FP, Claypool PL (1988) Chemical composition of old world bluestem grasses as affected by cultivar and maturity. J Range Manage 41:40-48

D'Antonio CM, Vitousek PM (1992) Biological invasion by exotic grasses, the grass-fire cycle, and global change. Annu Rev Ecol Syst 23:63-87

Daubenmire R (1959) A canopy-coverage method of vegetation analysis. Northwest Sci 33:43-64

Dutra HP, Barnett K, Reinhardt JR, Marquis RJ, Orrock JL (2011) Invasive plant specie alters consumer behavior by providing refuge from predation. Oecologia 166:649-657

Ellis EC, Ramankutty N (2008) Putting people in the map: anthropogenic biomes of the world. Front Ecol Environ 6:439-447

Fleharty ED, Mares MA (1973) Habitat preference and spatial relations of *Sigmodon hispidus* on a remnant prairie in west-central Kansas. Southwestern Nat 18:21-29

Fletcher RJ Jr, Koford RR (2002) Habitat and landscape associations of breeding birds in native and restored grasslands. J Wildlife Manage 66:1011-1022

Freund RJ, Wilson WJ (2003) Statistical methods, 2nd edn. Academic Press, San Diego, CA

Fuhlendorf SD, Townsend II DE, Elmore RD, Engle DM (2010) Pyric-herbivory to promote rangeland heterogeneity: Evidence from small mammal communities. Rangeland Ecol Manag 63: 670-678

Gannon WL, Sikes RS (2007) Guidelines of the American Society of Mammalogist for the use of wild animals in research. J Mammal 88:809-823

Gehring CA, Wolfe JE, Theimer TC (2002) Terrestrial vertebrates promote arbuscular mycorrhizal fungal diversity and inoculum potential in a rain forest. Ecol Lett 5:540-548

Hanchey MF, Wilkins KT (1998) Habitat associations of the small-mammal community in the Grand Prairie of north-central Texas. Tex J Sci 50:107-122

Harmoney KR, Hickman KR (2004) Comparing morphology of Caucasian old world bluestem and native grass. Agron J 96:1540-1544

Harmoney KR, Stahlman PW, Hickman KR (2007) Suppression of Caucasian old world bluestem with split application of herbicides. Weed Technol 21:573-577

Hayslett LA, Danielson BJ (1994) Small mammal diversity and abundances in three central Iowa grassland types. Prairie Nat 26:37-44

Heywood VH (1989) Patterns, extents, and modes of invasions by terrestrial plants. In: Drake JA, Mooney HA, di Castri F, Grovers RH, Kruger FJ, Rejmanek M, Williamson M (eds) Biological invasions: a global perspective. Wiley, Chichester, pp 31-60

Hickman KR, Farley GH, Channell R, Steier JE (2006) Effects of old world bluestem (*Bothriochloa ischaemum*) on food availability and avian community composition within the mixed-grass prairie. Southwest Nat 51:524-530

Hosmer DW, Lemeshow S (1989) Applied logistic regression. John Wiley and Sons, New York, NY

Jackman S (2012) pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory, Stanford University. Department of Political Science, Stanford University. Stanford, California, R package version 1.04.4

Jenks KE, Chanteap P, Damrongchainarong K, Cutter P, Redford T, Lynam AJ, Howard J, Leimgruber P (2011) Using relative abundance indicies from camera-trapping to test wildlife conservation hypotheses – an example from Khoa Yai National Park, Thailand. Trop Conserv Sci 4:113-131

Johnson DH (2006) Grassland bird use of conservation reserve program fields in the Great Plains. Fish and Wildlife Benefits of Farm Bill Programs: 2000-2005 Update, pp 17-32

Kantak GE (1996) Microhabitats of two *Peromyscus* (deer and white-footed mice) species in old fields and prairies of Wisconsin. Can Field Nat 110:322-325

Kaufman DW, Fineck EJ, Kaufman GA (1990) Small mammals and grassland fires. In: Collins SL, Wallace LL (eds) Fire in North American Grasslands. University of Oklahoma Press, Norman, OK, pp 46-79

Kaufman DW, Kaufman GA, Finck EJ (1995) Temporal variation in abundance of *Peromyscus leucopus* in wooded habitats of eastern Kansas. Am Midl Nat 133:7-17

Kirchner BN, Green NS, Sergeant DA, Mink JN, Wilkins KT (2011) Responses of small mammal and vegetation to a prescribed burn in tallgrass Blackland Prairie. Am Midl Nat 166:112-125

Kutner MH, Nachtsheim CJ, Neter J (2004) Applied linear regression models 4th edn. McGraw-Hill/Irwin, New York, NY

Liberton J, Burnham KP, Colbert J, Anderson DR (1992) Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecol Monogr 62:67-118

Litt AR, Steidl RJ (2011) Interactive effects of fire and nonnative plants on small mammals in grasslands. Wildlife Monogr 176:1-31

Lloyd JD, Martin TE (2005) Reproductive success of Chestnut-Collared Longspurs in native and exotic grassland. Condor 107:363-374

Maser C, Trappe JM, Nussbaum RA (1978) Fungal-small mammal interrelationships with emphasis on Oregon coniferous forest. Ecology 59:799-809

Matlack RS, Kaufman DW, Kaufman GA, McMillian BR (2002) Long-term variations in abundance of Elliot's short-tailed shrew (*Blarina hylophaga*) in tallgrass prairie. J Mammal 83:280-289

McCoy SD, Mosley JC, Engle DM (1992) Old world bluestem in western Oklahoma. Rangelands 14:41-44

Nolte KR, Fulbright TE (1997) Plant, small mammal and avian diversity following control of honey mesquite. J Range Manage 50:205-212

Odum EP (1955) An eleven year history of a Simodon population. J Mammal 36:368-378

Pearson DE, Ruggiero LF (2003) Transect versus grid trapping arrangements for sampling small-mammal communities. Wildlife Soc B 31:454-459

Pearson DE, Ortega YK, McKelevy KS, Ruggiero LF (2001) Small mammal communities and habitat selection in northern Rock Mountain bunchgrass: implications for exotic plan invasion. Northwest Sci 75:107-117

Perry RW, Thill RE (2005) Small-mammal responses to pine regeneration treatments in the Ouachita Mountains of Arkansas and Oklahoma, USA. Forest Ecol Manag 219:81-94

Phelps KL, McBee K (2009) Ecological characteristics of small mammal communities at a super fund site. Am Midl Nat 161:57-68

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. Bioscience 50:53-65

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273-288

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Redfearn DD (2007) Production and management of old world bluestems. Oklahoma Cooperative Extension Fact Sheet, F-3020:1-4

Reed HE, Seastedt TR, Blair JM (2005) Ecological consequences of C4 grass invasion of a C4 grassland: A dilemma for management. Ecol Appl 15:1560-1569

Rehmeier RL, Kaufman GA, Kaufman DW, McMillan BR (2005) Long-term study of abundance of the hispid cotton rat in native tall-grass prairie. J Mammal 86:670-676

Richards SA (2008) Dealing with overdispersed count data in applied ecology. J Appl Ecol 45:218-227

Robel RJ, Briggs JN, Dayton AD, Hulbert LC (1970) Relationships between visual obstruction measurements and weight of grassland vegetation. J Range Manage 23:295-298

Ruffner MA, McCulley RL, Nelson JA, Barnes TG (2012) Ecosystem function differs between old world bluestem invaded and native coastal prairie in south Texas. Biol Invasions 14:1483-1500

Sammon JG, Wilkins KT (2005) Effects of an invasive grass on grassland rodent community. Tex J Sci 57:371-382

Samson FB, Knopf FL, Ostlie WR (2004) Great Plains ecosystem: past, present, and future. Wildlife Soc B 32:6-15

Scheiman DM, Bollinger EK, Johnson DH (2003) Effects of leafy spurge infestation on grassland birds. J Wildlife Manage 67:115–121

Schmidt CD, Hickman KR, Channell R, Harmoney K, Stark W (2008) Competitive abilities of native grasses and non-native (*Bothriochloa spp*.) grasses. Plant Ecol 197:69-80

Schwartz OA, Whitson PD (1987) A 12-year study of vegetation and mammal succession on a reconstructed tallgrass prairie in Iowa. Am Midl Nat 117: 240-249

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture (2007) Web Soil Survey. http://websoilsurvey.nrcs.usda.gov/. Accessed 02 September 2013

Spyreas G, Wilm BW, Plocher AE, Ketzner DM, Matthews JW, Ellis JL, Heske EJ (2010) Biological consequences of invasion by reed canary grass (*Phalaris arundinacea*). Biol Invasions 12:1253-1267

Van Els P, Will RE, Palmer MW, Hickman KR (2010) Changes in forest understory with *Juniperus* in OK, USA. Appl Veg Sci 13:356-368

Venables WN, Ripley BD (2002) Modern Applied Statistics with S, 4th edn. Springer, New York

Vitousek PM, Mooney AH, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. Science 277:494-499

Watkinson AR, Ormerod SJ (2001) Grasslands, grazing and biodiversity: Editors' introduction. J Appl Ecol 38:233-237

Wiegert RG (1972) Avian versus mammalian predation on a population of cotton rats. J Wildlife Manage 36:1322-1327

Wilkins KT (1995) The rodent community and associated vegetation in a tallgrass Blackland Prairie in Texas. Tex J Sci 47:243-262

Wilson GWT, Hickman KR, Williamson MM (2012) Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. Mycorrhiza 22:327-336

Wilson MF (1993) Mammals as seed-dispersal mutualist in North America. Okios 67:159-176

White LM, Dewald CL (1996) Yield and Quality of WW-Iron Master and Caucasian bluestem regrowth. J Range Manage 49:42-45

Wolkovich EM, Bolger DT, Holway DA (2009) Complex responses to invasive grass litter by ground arthropods in a Mediterranean scrub ecosystem. Oecologia 161:697-708

Zeileis A, Kleiber C, Jackman S (2008) Regression Models for Count Data in R. J Stat Softw 27:1-25

TABLES

Table 1. Habitat variables used in analysis and development of habitat models for mammal species with greater than 30 captures (cotton rats and deer mice).

Variable	Units
Robel Height	dm
Litter Depth	mm
Percent Grass Cover	%
Percent of C_3 Species Cover	%
Percent of C_4 Species Cover	%
Percent of Forb Cover	%
Percent of Litter Cover	%
Percent of Bareground Cover	%
Percent of "Other" Cover	%
Percent of Shrub Cover	%
Percent of Standing Dead Plant Cover	%
Percent of Native Plant Species Cover	%
Percent of *B. ischaemum* Cover	%
Percent of Grass Cover at 5 cm	%
Percent of Grass Cover at 10 cm	%
Percent of Grass Cover at 25 cm	%
Percent of Grass Cover at 50 cm	%
Percent of Forb Cover at 5 cm	%
Percent of Forb Cover at 10 cm	%
Percent of Forb Cover at 25 cm	%
Percent of Forb Cover at 50 cm	%

Percent of Standing Dead Plant Cover at 5 cm	%
Percent of Standing Dead Plant Cover at 10 cm	%
Percent of Standing Dead Plant Cover at 25 cm	%
Percent of Standing Dead Plant Cover at 50 cm	%
Percent of Native Plant Species Cover at 5 cm	%
Percent of Native Plant Species Cover at 10 cm	%
Percent of Native Plant Species Cover at 25 cm	%
Percent of Native Plant Species Cover at 50 cm	%
Percent of *B. ischaemum* Cover at 5 cm	%
Percent of *B. ischaemum* Cover at 10 cm	%
Percent of *B. ischaemum* Cover at 25 cm	%
Percent of *B. ischaemum* Cover at 50 cm	%

Table 2. Common name, scientific name, and capture abundances of the eight mammal species captured during our two-year study (2011-2012) in Native and *B. ischaemum* invaded grasslands. These data were collected over 5,120 trap nights.

Common Name	**Scientific Name**	**Native (# of individuals)**	**B. ischaemum (# of individuals)**
Cotton Rat	*Sigmodon hispidus*	74	237
Deer Mouse	*Peromyscus maniculatus*	101	36
Eastern Harvest Mouse	*Reithrodontomys humulis*	4	1
Elliot's Short-tailed Shrew	*Blarina hylophaga*	3	10
Fulvous Harvest Mouse	*Reithrodontomys fulvescens*	4	1
Hispid Pocket Mouse	*Chaetodipus hispidus*	1	0
Prairie Vole	*Microtus ochrogaster*	4	7
White-footed Mouse	*Peromyscus leucopus*	2	2
Total		193	294

Table 3. Candidate models having an AICcω ≥ 0.01 for species with greater than 30 captures during our two-year field study (2011-2012) with corresponding K values, AICc scores, changes in AICc scores, and Akaike weights (AICcω).

Species	**Model**	**K**	**AICc**	**ΔAICc**	**AICcω**
Cotton Rat	–Bareground +*B. ischaemum* Cover at 50 cm	4	437.9	0.0	0.8942
Cotton Rat	+*B. ischaemum* Cover at 50 cm	3	442.6	4.7	0.0846
Cotton Rat	–Bareground + Total Grass Cover at 50 cm	4	445.4	7.5	0.0212
Deer Mouse	–Total Grass Cover	3	333.5	0.0	0.3034
Deer Mouse	+DB_Litter –B. ischaemum Cover	4	334.1	0.6	0.2291
Deer Mouse	$-C_4$ Species Cover	3	335.4	1.8	0.1207
Deer Mouse	–Litter Depth +Litter Cover	4	336.5	3.0	0.0694
Deer Mouse	+Litter Cover	3	336.5	3.0	0.0687
Deer Mouse	–*B. ischaemum* Cover	3	337.3	3.8	0.0445
Deer Mouse	–*B. ischaemum* Cover at 5 cm	3	337.6	4.1	0.0399
Deer Mouse	–*B. ischaemum* Cover at 10 cm	3	337.6	4.1	0.0393
Deer Mouse	–Robel Height	3	337.7	4.2	0.0376
Deer Mouse	–Total Grass Cover at 5 cm	3	338.5	5.0	0.0254
Deer Mouse	–*B. ischaemum* at 25 cm	3	338.8	5.2	0.0220

FIGURES

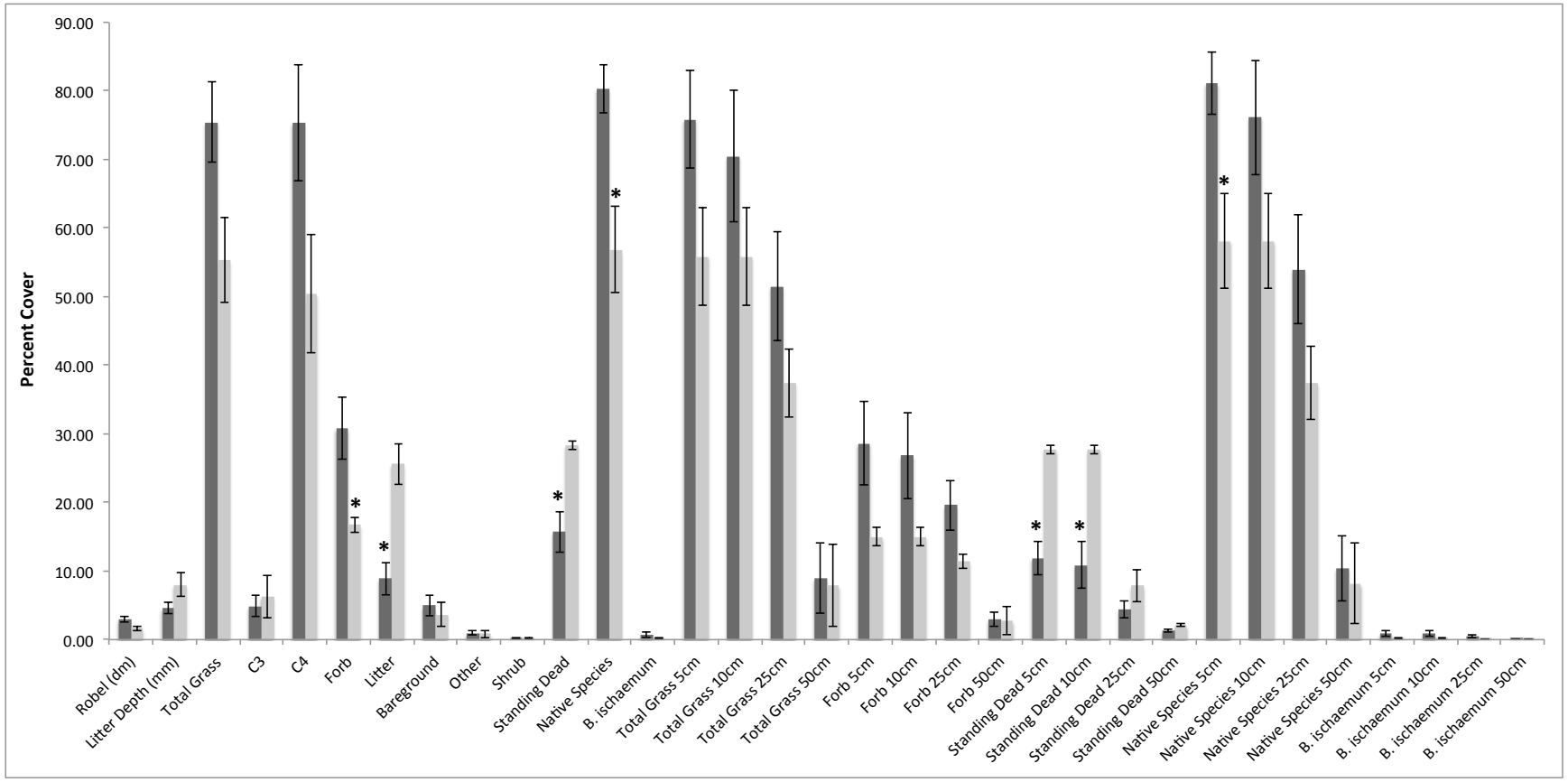


Figure 1.

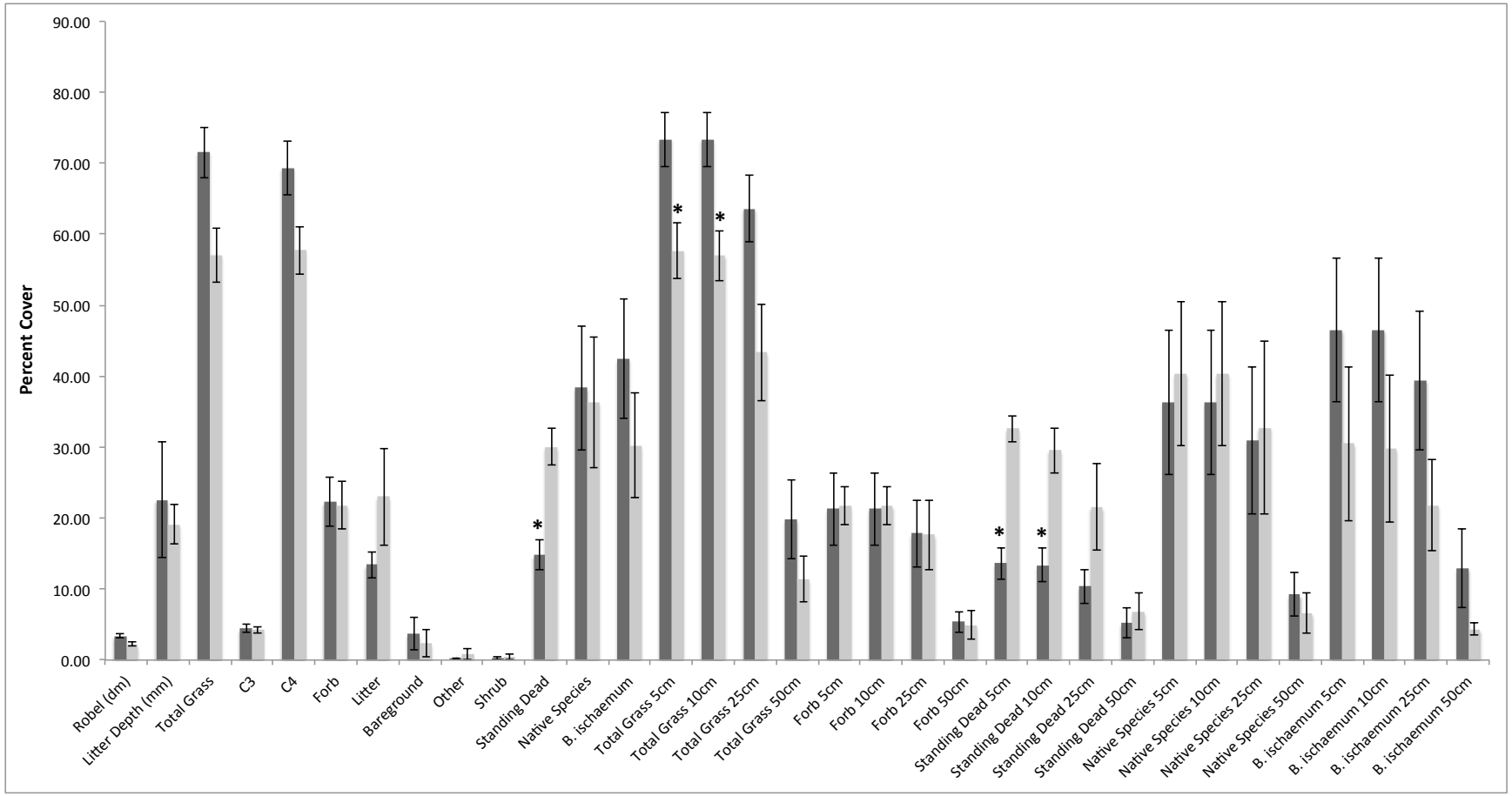
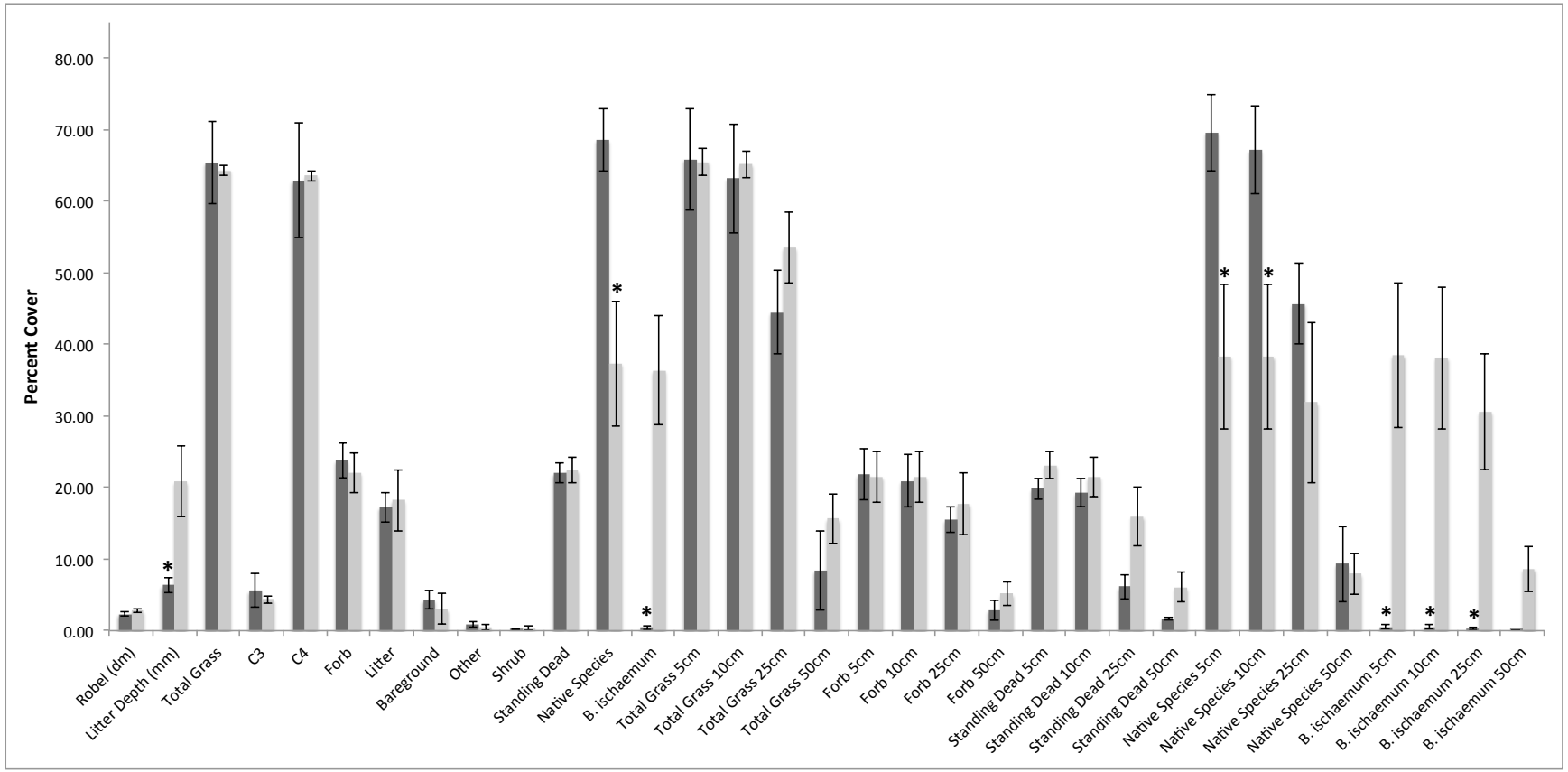

Percent Cover
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Robel (dm)
Litter Depth (mm)
Total Grass
C3
C4
Forb
Litter
Bareground
Other
Shrub
Standing Dead
Native Species
B. ischaemum
Total Grass 5cm
Total Grass 10cm
Total Grass 25cm
Total Grass 50cm
Forb 5cm
Forb 10cm
Forb 25cm
Forb 50cm
Standing Dead 5cm
Standing Dead 10cm
Standing Dead 25cm
Standing Dead 50cm
Native Species 5cm
Native Species 10cm
Native Species 25cm
Native Species 50cm
B. ischaemum 5cm
B. ischaemum 10cm
B. ischaemum 25cm
B. ischaemum 50cm

Figure 2.


Percent Cover
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Robel (dm)
Litter Depth (mm)
Total Grass
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Forb 10cm
Forb 25cm
Forb 50cm
Standing Dead 5cm
Standing Dead 10cm
Standing Dead 25cm
Standing Dead 50cm
Native Species 5cm
Native Species 10cm
Native Species 25cm
Native Species 50cm
B. ischaemum 5cm
B. ischaemum 10cm
B. ischaemum 25cm
B. ischaemum 50cm

Figure 3.

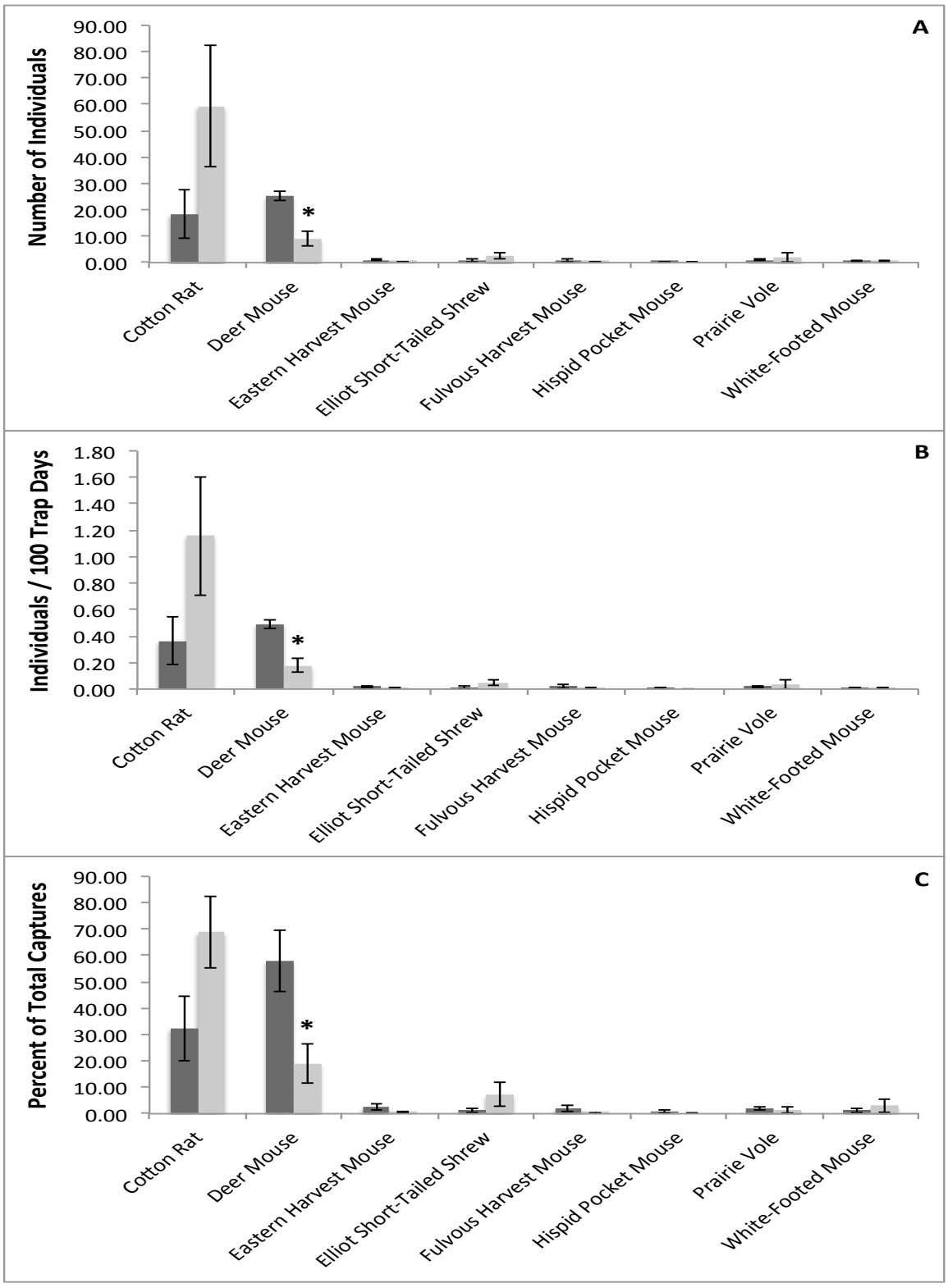
A
Number of Individuals
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
*
Cotton Rat
Deer Mouse
Eastern Harvest Mouse
Elliot Short-Tailed Shrew
Fulvous Harvest Mouse
Hispid Pocket Mouse
Prairie Vole
White-Footed Mouse
B
Individuals / 100 Trap Days
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
*
Cotton Rat
Deer Mouse
Eastern Harvest Mouse
Elliot Short-Tailed Shrew
Fulvous Harvest Mouse
Hispid Pocket Mouse
Prairie Vole
White-Footed Mouse
C
Percent of Total Captures
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
*
Cotton Rat
Deer Mouse
Eastern Harvest Mouse
Elliot Short-Tailed Shrew
Fulvous Harvest Mouse
Hispid Pocket Mouse
Prairie Vole
White-Footed Mouse

Figure 4.

FIGURE CAPTIONS

Figure 1: 2011 (black filled bars) and 2012 (gray shaded bars) vegetative composition and structure of native trapping sites. Asterisk (*) above bar indicate significant reduction (alpha = 0.05) between years. Error bars represent 1 SE. “Other” is a compilation of ground cover consisting of rocks, sticks, manure, etc.

Figure 2: 2011 (black filled bars) and 2012 (gray shaded bars) vegetative composition and structure of *B. ischaemum* invaded trapping sites. Asterisk (*) above bar indicate significant reduction (alpha = 0.05) between years. Error bars represent 1 SE. “Other” is a compilation of ground cover consisting of rocks, sticks, manure, etc.

Figure 3: Native sites (black filled bars) and *B. ischaemum* invaded sites (gray shaded bars) vegetative composition and structure of grasslands surveyed during 2011-2012. Asterisk (*) above bar indicate significant reduction (alpha = 0.05) between habitat types. Error bars represent 1 SE. “Other” is a compilation of ground cover consisting of rocks, sticks, manure, etc.

Figure 4: The (a) raw abundances, (b) abundance index, and (c) relative abundance of the eight small mammal species captured in native sites (black filled bars) and *B. ischaemum* invaded sites during the 2011-2012 seasons. Asterisk (*) above bar indicate significant reduction (alpha = 0.05) between habitat types. Error bars represent 1 SE.

VITA

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- Soil Ecological Society
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